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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **277th Meeting** *of the Biochemical Society was held in the Department of Chemistry, The University, Nottingham, on Friday* 17 *June* 1949, *when the following papers were read:*

COMMUNICATIONS

Separation of the Various Penicillins by Partition Chromatography. By F. Dobson and S. W. Stroud. (*Biochemistry Division, Research Department, Messrs Boots Pure Drug Co. Ltd., Nottingham*)

Various authors have previously described methods for the separation of the various penicillins based on the silica-gel partition chromatogram of Martin & Synge (1941). Catch, Cook & Heilbron (1942) used a modified silica-gel chromatogram for the purification of crude penicillin, but this was capable of little development. Levi (1948) dealt with the theoretical aspects and the use of a silica-gel partition chromatogram, and Boon, Calam, Gudgeon & Levi (1948) described a method for the analysis of crude penicillin using this type of column. Fischbach, Eble & Mundell (1947) also described the separation of the various penicillins by means of a silica-gel/phosphate buffer partition chromatogram, in which a high degree of separation occurred, but omitted to give the precise details of the method. All these methods used silica gel as the absorbent, the silica having to be extremely carefully prepared and standardized to obtain reproducible results (Harris & Wick, 1946).

In the course of the present investigations it has been found that efficient separation of the various penicillins can be effected by the use of a kieselguhr/sodium citrate buffer partition chromatogram, no pre-treatment of the kieselguhr being necessary. Optimum conditions were established with 40% (v/v) saturated sodium citrate buffer solution of pH 5·7 as the stationary phase, ether being used as the mobile phase. Under these conditions the separated penicillins can be rapidly eluted from the column, and we have been able to prepare 500–1000 mg. quantities of the various penicillins in a pure state from 4 to 6 g. of commercial samples of mixed penicillins on a 500 g. kieselguhr/250 ml. sodium citrate buffer column. The precise loading on the column is dependent on the relative proportions of the various penicillins in the sample.

Small variations in pH have a considerable effect on the efficiency of the separations, and increasing the concentration of the buffer above the established optimum decreases the effective length of the column.

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Acetic Acid in Bovine Peripheral Blood and its Utilization by the Mammary Gland. By G. L. McClymont (Walter and Eliza Hall Veterinary Research Fellow, University of Sydney) (introduced by R. Scarisbrick). (*Institute of Animal Pathology, University of Cambridge*)

An arteriovenous difference of volatile fatty acids on the head of the sheep was demonstrated by Reid (1949). Work is now reported on the volatile fatty acid metabolism of bovines with special reference to arteriovenous differences on the mammary gland.

Arterial blood was obtained by rectal puncture of the internal iliac (Graham, Kay & McIntosh, 1936), or from carotid loop established in bovines by a modification of the operation described for goats by Graham, Turner & Gomez (1937). Volatile fatty acid was isolated from arterial blood, by the method of Friedemann (1938) using a metaphosphoric acid filtrate. The method of partition chromatography described by Moyle, Baldwin & Scarisbrick (1948) was used for identification and estimation of the acids. With diets of grass hay and concentrates, the

volatile fatty acid was found in five cows to be over 91 % (molar basis) acetic, with small proportions of propionic, butyric, and at least two higher acids. For most purposes the volatile fatty acid in bovine peripheral blood can therefore be regarded and calculated as acetic. Reid (1948), using the method of Elsden (1946), found 100 and 96 % acetic acid in two blood samples from sheep.

Volatile fatty acid, calculated as acetic, was routinely estimated by a modification of the method of McClendon (1944). In three cows studied, acetic acid levels in arterial blood usually reached a maximum within 2–4 hr. of feeding, typically attaining levels of 8–12 mg./100 ml. of blood, declining to 3–6 mg./100 ml. 24 hr. after feeding and to 1·5–3 mg./100 ml. 48 or more hours after feeding.

Both the non-lactating and lactating mammary gland removed acetic acid from the blood, the arteriovenous difference of the lactating gland in the fed animal usually being from 40 to 80 % of the arterial level or 2–6 mg./100 ml. of blood.

These *in vivo* findings are in accord with the *in vitro* work of Folley & French (1949) on ruminant mammary gland tissue slices.

An association was found between the decline in the Reichert Meissl value of milk fat in starvation, as described by Smith & Dastur (1940), and the fall in the arteriovenous difference of acetic acid, using oxytocin injections to empty the udder as completely as possible at each milking. However, neither intraruminal infusion of acetic acid nor intravenous infusion of sodium acetate affected the starvation-lowered R.M. value. This confirms the findings of Mann & Shaw (1947) and Folley & Malpress (cited by Folley & French, 1949), but the explanation suggested by Folley & French, that adequate blood levels of acetate were not obtained, was found invalid for these experiments.

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Microbiological Assay of Growth Factors Essential for *Lactobacillus leichmanii* 313 and *Leuconostoc citrovorum*. By K. A. Lees and W. B. Emery. (*Fermentation Division, Glaxo Laboratories Ltd., Barnard Castle*)

Microbiological tube assays for the determination of Smith's A.P.A.F. and other growth factors using *Lactobacillus leichmanii* and *Leuconostoc citrovorum* as test organisms have been developed.

Lac. leichmanii is maintained by daily transfer in liquid medium (yeast extract 1 %, tomato juice 5 %, skimmed milk *ad* 100 %). *Leu. citrovorum* is maintained in stab cultures (glucose 0·5 %, yeast extract 0·3 %, agar 1·5 %) by fortnightly transfer.

Lac. leichmanii inoculum is prepared by two daily transfers in basal assay medium plus 0·2 % Examen. The second transfer is centrifuged, the cells washed once with saline and resuspended to B.W. opacity 4. This suspension is diluted 1/8 in a mixture of equal volumes of saline and clarified tomato juice (sterilized by filtration). 0·5 ml. of this inoculum is used per tube. *Leu. citrovorum* inoculum is produced similarly from a single 24 hr. transfer from the stab culture into the supplemented basal medium. This is made to opacity 4/8 in saline only.

The basal medium used for both organisms is that of Snell, Kitay & McNutt (1948), modified by omission of enzymatic casein digest and reduction of the level of salts B. To prevent precipitation in the tubes and consequent high blanks, the medium is preheated in bulk and filtered hot. 6 × 1 in. tubes are used, each tube containing 8 ml. of medium. Tubes are autoclaved at 15 lb. pressure for 10 min. Incubation is normally carried out at 37° for 16 hr. and turbidity measured on a Spekker absorptiometer. Maximum response of *Lac. leichmanii* is obtained with 0·005 μg. of Smith's crystalline factor per tube. Maximum response of *Leu. citrovorum* is obtained with 1 ml. of 1 mg./ml. Examen per tube. Both factors are sterilized by filtration and added aseptically to the tubes.

Leu. citrovorum does not respond to 2000 times the level of Smith's factor required to stimulate *Lac. leichmanii*.

Both factors have been found to be present in refined liver extracts and in concentrates prepared from fermentation residues, shown to be clinically active in pernicious anaemia. The *Leu. citrovorum* factor appears to be more stable to heat than the *Lac. leichmanii* factor. Stability tests on Examen at 37° indicate that the *Lac. leichmanii* factor is labile at alkaline pH and the *Leu. citrovorum* factor is labile at acid pH.

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The Influence of Dietary Protein Levels on Experimental Liver Injury in Dogs. By C. J. Bardawill and A. G. Gornall. (*Department of Pathological Chemistry, University of Toronto*)

For many years patients with liver disease received diets rich in carbohydrate, but low in protein and fat. About 10 years ago, as a phase in the elucidation of the lipotropic food factors, the value of additional dietary protein was noted and became emphasized. Patek & Post (1941) reported very encouraging results in the treatment of cirrhosis, using a generous diet containing 140 g. of protein, with added B vitamins, and liver extract injections. This work initiated a rapid trend to high-protein dietary regimes in liver disease, intakes of 200–300 g. a day being advocated by Morrison (1947) and others. From this one might infer that experimental hepatic damage should tend to be more marked and heal less rapidly in animals kept on a relatively low protein diet.

Carbon tetrachloride has been administered twice weekly by stomach tube to two small groups of adult dogs maintained on isocaloric diets containing approximately 17 and 70 g. of protein per day respectively. After 50–75 doses of the toxin early cirrhotic changes were present in all of the animals, but no distinction could be drawn between the two groups, and it was decided to repeat the experiment on the same animals under more closely comparable conditions. After a convalescent period the animals were placed again on the different diets, given sixteen consecutive doses of carbon tetrachloride and allowed to recover. Following a further rest period the groups were reversed as to diet and the toxin administration repeated.

The results, measured by the bromsulfalein excretion test, the serum alkaline phosphatase, and the serum albumin and globulin concentrations, showed during and after the first sixteen doses of toxin no appreciable difference between the group on the lower and higher protein intake. Individual responses to the carbon tetrachloride were, however, quite varied. On reversing the two groups it was again impossible to draw any clear distinction between the effects of the toxin. Most notable was the similarity in the response of each individual animal on the two different diets.

The experimental results are regarded as lending support to the view that a large amount of protein (*per se*) is of doubtful benefit in the diet of patients with chronic liver disease. A nutritious diet containing moderate amounts of first-class protein (supplemented possibly with certain accessory food factors) may indeed be entirely adequate and perhaps best.

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The Conversion of Acetate to Glycine in the Rat. By H. R. V. Arnstein and A. Neuberger. (*The National Institute for Medical Research, London, N.W. 3*)

Adult rats (Albino Institute strain) were administered sodium acetate labelled with ^{14}C on the methyl and on the carboxyl groups respectively. The rats were given at the same time DL-α-amino-γ-phenylbutyric acid and sodium benzoate. Hippuric acid and L-α-acetamido-γ-phenylbutyric acid were isolated from the urine by counter-current extraction with chloroform and ethyl acetate and their radioactivity determined (Table 1).

The activities of the hippuric acid show that both carbon atoms of acetate appear in the glycine molecule, though the specific activity of the hippuric acid is very much smaller than that of the acetamido compound.

Table 1. *All counts were determined with infinite thickness samples (20 mg. or more of substance per cm.²) on a 1·2 cm.² disk. Under these conditions, a substance with a specific activity of 1 μc./mg. gave 10^6 counts/min./mg.*

Rat no.	Counts/min./mM. L-α-Acetamido-γ-phenylbutyric acid	Hippuric acid	Xanthydryl urea	Diluted hippuric acid	Formaldehyde dimedon	$BaCO_3$ from carboxyl group
7*, 8*	$6{\cdot}25 \times 10^5$	$1{\cdot}325 \times 10^4$	$4{\cdot}05 \times 10^4$	$3{\cdot}5 \times 10^3$	No activity	$3{\cdot}48 \times 10^3$
9†	$3{\cdot}0 \times 10^5$	$1{\cdot}25 \times 10^4$	$2{\cdot}29 \times 10^4$	$4{\cdot}0 \times 10^3$	$2{\cdot}45 \times 10^3$	$1{\cdot}18 \times 10^3$
10‡	$2{\cdot}25 \times 10^5$	$2{\cdot}18 \times 10^4$	$3{\cdot}82 \times 10^4$			
11§, 12§	$4{\cdot}03 \times 10^5$	$1{\cdot}51 \times 10^4$	Not isolated	$1{\cdot}51 \times 10^4$‖	$2{\cdot}85 \times 10^3$	$2{\cdot}02 \times 10^3$

* Given 22·6 μc. $CH_3C^{14}O_2Na$ orally.
† Given 18·75 μc. $C^{14}H_3CO_2Na$ parenterally in three equal doses at two-hourly intervals.
‡ Given 18·75 μc. $C^{14}H_3CO_2Na$ parenterally in a single dose.
§ Given 18·75 μc. $C^{14}H_3CO_2Na$ orally.
‖ This sample was diluted at the glycine stage.

The hippuric acid was hydrolysed and the isolated glycine degraded with ninhydrin; the formaldehyde was precipitated as the dimedon derivative and the CO_2 as $BaCO_3$. The carboxyl carbon atom of acetate is transformed exclusively into the carboxyl carbon of glycine, no activity appearing in the dimedon derivative. With methyl-labelled acetate activity was found in both fractions, although the specific activity was always higher in the methylene group (Table 1). These findings are compatible with the interpretation that acetate enters the tricarboxylic acid cycle, is converted into pyruvic acid and is then transformed into serine by reversal of the reaction studied by Chargaff & Sprinson (1943). The serine is then degraded to glycine (Shemin, 1946). The relatively low activity of the hippuric acid is explained by the fact that most of the glycine used for conjugation with benzoic acid is obtained from the free glycine present in tissues, glutathione and proteins. It is suggested that the synthesis from acetate represents a major pathway for the synthesis of glycine from nitrogen-free precursors.

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Studies on Porphyrin Metabolism in the Rabbit. By J. S. F. Niven and A. Neuberger. (*National Institute for Medical Research, London, N.W. 3*)

Adult rabbits were given 31·5 atom % excess ^{15}N, usually over a period of 1–3 days. Blood samples were collected at intervals and crystalline haemin prepared. Some of the animals also received small doses of sodium benzoate, and hippuric acid was isolated. The results show that the isotope appears in the haemin within a day or less after the administration of glycine has started. On a high protein diet the isotope content of the haemin reached the maximum value (about 0·2 %) about one day after the administration of glycine had stopped. The values of the ^{15}N content of the newly formed haemin and of the hippuric acid excreted during the same period appear to be almost identical in rabbits kept on the high protein diet. On a low protein diet the ^{15}N content of the haemin rose to almost 0·4 % and, under these conditions, the isotope content of the haemin continued to rise for some days after the administration of glycine had been stopped. The ^{15}N content of the hippuric acid excreted by rabbits on the low protein diet was markedly lower than the calculated ^{15}N content of the newly formed haemin, suggesting that under such conditions, the hippuric acid is largely formed from glycine fractions of lower isotope content than those used for the formation of porphyrins.

The life-span of the red cell in the normal rabbit was found to vary between 60 and 65 days.

In rabbits made anaemic either by bleeding or by acetyl phenylhydrazine until the haemoglobin content was reduced to about 30 %, porphyrin incorporation into the red cells was greatly increased, as shown by the ^{15}N results. Differential centrifugation showed that the ^{15}N content in th ereticulocytes was particularly high. However, most of the cells formed in the recovery period appear to have a much shorter life-span than cells formed under normal conditions.

Effects of Electrolytes on the Molecular Weight of Tropomyosin. By G. S. Adair, K. Bailey and T. C. Tsao. (*Biochemical Laboratory, and Low Temperature Research Station, University of Cambridge*)

The molecular weight of tropomyosin (TM) from rabbit skeletal muscle dissolved in a salt solution was found to be 90,500 by the methods of osmotic pressure, sedimentation-diffusion and amino-acid analysis (Bailey, Gutfreund & Ogston, 1948). In salt-free solutions TM aggregates into large fibrils (Bailey, 1948; Astbury, Reed & Spark, 1948) which disaggregate again on addition of salt. In order to determine the effects of salts on the degree of aggregation of tropomyosin, the mol.wt. of TM has therefore been reinvestigated by osmotic pressure measurements, employing the toluene and tetrachloroethylene osmometers of Adair (unpublished), and for the calculation of results the extrapolation procedure of Adair & Robinson (1930). In Table 1 are listed the values obtained (*a*) in neutral salt solutions of varying ionic strength, (*b*) in concentrated urea solutions, and (*c*) in acid solution (pH 2). molecular weight deduced from the histidine content, viz. $3 \times 18{,}180 = 54{,}540$. The higher molecular weights in the other series must be considered average values for mixtures of single, double and multi-molecular species. With the exception of protein recovered from urea solutions the TM in all series could be crystallized.

Previous work with the ultracentrifuge (Bailey, Gutfreund & Ogston, 1948) has shown that solutions of TM which are now considered to contain several molecular species in varying states of aggregation give a single sedimenting boundary. This behaviour seems characteristic of systems containing markedly asymmetric particles (Campbell & Johnson, 1944).

Another interesting feature is the high degree of osmotic pressure-concentration dependence. This can be seen from the values of ϕ in Table 1, where ϕ is a coefficient representing the effects of all factors

Table 1. *Particle weights of tropomyosin*

(C_v=g. protein/100 ml. solvent.)

Buffer	pH	Ionic strength	Particle wt.	ϕ	
				$C_v=1$	$C_v=3$
Phosphate (0·06 M)	6·5	0·10	135,000	1·40	2·60
Phosphate (0·06 M) + NaCl (0·1 M)	6·5	0·20	112,000	1·43	2·88
Phosphate (0·04 M) + KCl (0·2 M)	6·5	0·267	88,000*	1·14	1·44
Phosphate (0·06 M) + NaCl (0·2 M)	6·5	0·30	72,000	1·17	1·51
Phosphate (0·06 M) + NaCl (0·5 M)	6·5	0·60	67,000	1·22	1·66
Phosphate (0·03 M) + NaCl (1·05 M)	6·5	1·10	65,000	1·21	1·62
Phosphate (0·075 M) + urea (6·67 M)	6·5	0·1	53,000	1·73	3·24
KCl (0·09 M) + HCl (0·01 M)	2·1	0·1	51,000	1·37	2·84

* Data of Bailey *et al.* (1948).

In phosphate buffers (pH 6·5) the mol.wt. falls exponentially with increase of ionic strength, approaching asymptotically the values of 51,000 and 53,000 obtained in acid and urea respectively. The mean value conforms with that based on the minimal causing deviation from the simple form of van't Hoff's law. Even in buffers of high salt concentration, where the Donnan effect is small, the values of ϕ are still high compared with those of other proteins (cf. Adair & Robinson, 1930).

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Separation of *N*-2:4-Dinitrophenyl Derivatives of the Methyl Esters of Amino-acids by Adsorption Chromatography. By A. G. LOWTHER and W. S. REITH (REICH). (*Department of Organic Chemistry, The University, Leeds* 2)

N-2:4-Dinitrophenyl-derivatives of amino-acids were first prepared by Abderhalden & Blumberg (1910). Sanger (1945) applied partition chromatography to the separation of mixtures of these coloured derivatives.

It has been found previously (Reich, 1948) that mixtures of the methyl esters of *N*-azobenzene-*p*-sulphonyl-derivatives of some amino-acids can be separated quantitatively by adsorption chromatography on alumina-columns. We have now prepared the methyl esters of the *N*-2:4-dinitrophenyl-derivatives of certain amino-acids and find that they can be selectively adsorbed on suitably prepared alumina; a mixture of such derivatives can be resolved by the development of the chromatogram, and, after elution, the unchanged methyl esters can be quantitatively recovered and identified.

The alumina for adsorption was prepared by heating aluminium oxide with a 10% solution of acetic acid in methanol, washing thoroughly with methanol and drying, first at room temperature and then at 40° for 24 hr.

A mixture of 37 mg. of *N*-2:4-dinitrophenyl-glycine methyl ester (m.p. 124°) and 43 mg. of *N*-2:4-dinitrophenyl-DL-alanine methyl ester (m.p. 121°) dissolved in benzene (8 ml.) was adsorbed on a column formed from a slurry of alumina (130 g.) and a solution of 1 vol. of benzene in 3 vol. of ligroin (b.p. 60–80°) (250 ml.). When all the solution of the esters had been added to the column the chromatogram was developed with 400 ml. of the benzene/ligroin solution. Finally each band was removed, and the adsorbed compounds eluted with a mixture of acetone/methanol (1:1).

After evaporation of the acetone/methanol *in vacuo*, the residue was dissolved in 20 ml. of benzene, the solution filtered through a glass filter (G. 4), and the benzene evaporated *in vacuo*.

The appearance of the final chromatogram, and the weights of the recovered compounds were as follows:

13 mm. colourless 34 mm. yellow	34·8 mg. *N*-2:4-dinitrophenyl-glycine methyl ester, m.p. 124–125°.
13 mm. colourless 23 mm. yellow	42·7 mg. N-2:4-dinitrophenyl-DL-alanine methyl ester, m.p. 121–122°.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **278th Meeting** *of the Biochemical Society was held in the Biochemistry Department, University College, Dundee, on Friday,* 29 *July* 1949, *when the following papers were read:*

COMMUNICATIONS

Nucleic Acid Metabolism of *Escherichia coli*. By MARJORY STEPHENSON (the late) and JENNIFER M. MOYLE (introduced by E. F. GALE). (*Medical Research Council Unit for Chemical Microbiology, Biochemical Laboratory, University of Cambridge*)

A preliminary study of the nucleic acid metabolism in a strain (*H*) of *Esch. coli* has been carried out using washed suspensions of cells which had been grown on a tryptic digest of casein with 0·5% glucose for 22 hr. at 37°.

The general phosphorus distribution in the cells was investigated, using Fiske & Subbarow's (1925) method for estimating inorganic orthophosphate. Acid-insoluble P amounted to 15–16 μg./mg. cells (dry wt.), and acid-soluble to 1 μg./mg. cells (of which approximately 45% was inorganic and 55% organic P).

On incubating the cell suspension in bicarbonate buffer (pH 7·4) at 37° in the presence of 0·2 M-Na lactate for 1 hr., the P distribution underwent a marked change. The cells lost 50% of their total P (invariably from the acid-insoluble fraction only), all of which appeared in the supernatant, 60% of this being organic and 40% inorganic P.

The acid-insoluble fraction of the cells was treated according to the method of Schmidt & Thannhauser (1945) to separate the two nucleic acids. Ribonucleic acid (RNA) amounted to 10–12·2 μg. P/mg. cells and deoxyribonucleic acid (DRNA) to 2·7–3·2 μg. P/mg. cells. This gives a value of 15% (±5%) for the total nucleic acid. After incubation as above for 3 hr. the RNA decreased by 60% and the DRNA by 20–33%.

The organic P fraction appearing in the supernatant was fractionated with barium acetate at pH 8·2, and 90% of the total organic P was found in the 'barium soluble-alcohol insoluble' fraction. The molar ratio of pentose/P in this fraction was 1:2·4, and in the 'barium-insoluble' fraction 1:1·7. Both fractions were completely hydrolysed in N-HCl at 100° in 10 min.

No degradation of nucleic acids could be demonstrated in the presence of glucose. The action of Na lactate could, however, be duplicated by equivalent concentrations of NaCl and KCl, though $MgCl_2$ completely inhibited the breakdown of nucleic acids. Greenstein, Carter & Chalkley (1947) have shown that the rate of breakdown of Na salts of nucleic acids by aqueous animal tissue extracts is very greatly increased in the presence of salts, but here Mg is reported to be relatively more effective than Na.

The breakdown of RNA in intact cells was apparently inhibited almost completely by the addition of inorganic phosphate (40 μg. P/mg. cells) and ammonium sulphate (10 mg. N-NH_3/100 mg. cells) to the reaction mixture. In this case, a source of energy was required, since replacement of Na lactate by NaCl gave no inhibitory effect.

That cell suspensions previously boiled 30 min. underwent no change in nucleic acids in the presence of salts suggested that these changes were enzymatically controlled. Acetone powders prepared from suspensions of crushed cells possessed activity similar to that of intact cells, and extraction of the acetone powders with buffers gave cell-free extracts which were active towards RNA (and sometimes towards DRNA). The rate of breakdown of the RNA of boiled suspensions of crushed cells by such extracts was linear for the initial 20 min. and was optimal at pH 7·0.

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The Configuration at C-20 of the Urinary Pregnane-3(α):17(α):20-triol of Butler and Marrian.
By W. KLYNE. (*Postgraduate Medical School, London, W.* 12)

Butler & Marrian (1937, 1938) isolated from the urine of patients with adrenal virilism a pregnane-3(α):17(α):20-triol. Subsequently, Mason & Kepler (1945) isolated from similar material a compound which was apparently the same triol. Mixed melting-point determinations on the triols and their diacetates, kindly supplied by Prof. G. F. Marrian, F.R.S., and Dr H. L. Mason, have shown that the two triols are identical. All the following melting points are corrected. Triol diacetates: Marrian (purified by chromatography), 155–7°; Mason, 157–9°; mixture, 155–8°. Triols: Marrian (by hydrolysis of the diacetate), 247–9°; Mason, 246–9°; mixture, 246–8°.

The configuration of the urinary triol at C-20 has not been discussed previously. It has now been shown by means of the method of molecular rotation differences that the triol is a 20α-hydroxy compound. (All configurations at C-20 in this paper are allotted following the convention of Fieser & Fieser, 1948.)

The optical rotation of the triol quoted by Mason & Kepler ($[M]_D - 13 \pm 6°$) was compared with those calculated for the 20α and 20β isomers from the rotations of the analogous compounds in the 3(β)-hydroxy*allo*pregnane series (compounds O and J, Steiger & Reichstein, 1938), using the molecular rotation difference tables of Barton & Klyne (1948). The comparison (Table 1A) gave no conclusive answer, since the calculated $[M]_D$ values for the triols differ only by about 15°; it appeared that the urinary triol was probably the 20α compound.

Table 1. *Molecular rotations*

(Solvents are indicated by formulae in brackets. Values in italics are calculated from the tables of Barton & Klyne, 1948.)

Compound	$[M]_D$ *allo*Pregnane-3(β):17(α):20-triol	$[M]_D$ Pregnane-3(α):17(α):20-triol
	A. Triols	
20α isomer	−42° (MeOH)	*−4°*
20β isomer	−27° (EtOH)	*+11°*
Urinary triol		−13° (EtOH)
	B. Triol diacetates	
20α isomer	−126° (Me_2CO)	*+23°*
20β isomer	+103° (Me_2CO)	*+252°*
Urinary triol diacetate		+3° ($CHCl_3$)

The calculated $[M]_D$ values for the triol diacetates, however, differ by about 230° (Table 1B), and the configuration of the urinary triol at C-20 could thus be allotted with certainty, using the $[M]_D$ value of its diacetate. The rotation of Prof. Marrian's triol diacetate was therefore determined:

$$[\alpha]_D^{23°} + 0{\cdot}8 \pm 0{\cdot}8° \ (c = 1{\cdot}5 \text{ in chloroform}),$$

$[M]_D + 3 \pm 3°$. This value, when compared with the calculated values for the isomeric diacetates, shows clearly that the urinary triol is

pregnane-3(α):17(α):20α-triol.

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Iron Metabolism in Hens' Eggs during Development of the Embryo.
By W. N. M. RAMSAY. (*Department of Biochemistry University of Edinburgh*)

Sendju (1927) determined haemoglobin in 7-, 14- and 19-day chick embryos by the Sahli acid haematin method after washing the pigment out of the vessels. Schønheyder (1938), in a more extensive investigation, used the rough pyridine haemochromogen technique developed for yeast by Anson & Mirsky (1929). McFarlane & Milne (1934) determined Fe in the chick embryo and its liver, but their technique required them to discard the highly vascular yolk-sac.

In the present work haem-pigment synthesis has been indirectly estimated in incubated, frozen, homogenized eggs from the difference between total Fe (modified from Ramsay, 1944) and 'non-haem' Fe, defined as Fe reacting with 2-2′-dipyridyl in the presence of NH_2OH as reducing agent (Hill, 1931; Sherman, Elvehjem & Hart, 1934; Jones, 1948). The total Fe of 101 eggs averaged 1·07 mg./egg (S.D. ± 0·20 mg., distribution approximately normal). In twelve unincubated eggs 98% (range 95–101%) of the total Fe was found to be 'non-haem'. The technique as used at present is thus not sensitive

enough to follow the small but definite synthesis of Hb which takes place during the early days of incubation. Haem-Fe, however, increases smoothly and steadily from about 0·08 mg./egg (8% of total) at 8 days to 0·45 mg. (40% of total) at 14 days and 0·8–1·1 mg. (64–75% of total) at 19–21 days. The amount of haem-Fe towards the end of incubation appears to depend largely on the total Fe. The figures quoted for haem-Fe may be used to calculate Hb values, which seem about 50% greater than those of Schønheyder, and much greater than those of Sendju.

If haem-Fe is compared with embryo weight (figures of Byerly (1932), compiled from over 2000 eggs), it appears that during the middle period of incubation the rate of haem synthesis is relatively greater than that of increase in gross body weight (wet or dry). Thus, at 14 days, the embryo weight is about 35% of its final value, whereas 50% of the final amount of haem has been synthesized. This is doubtless related to the existence and growth of the important extra-corporeal circulation used by the embryo chick for both its respiration and its nutrition.

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Acid-soluble Phosphorus in Hens' Eggs during Development of the Embryo. By W. N. M. Ramsay. (*Department of Biochemistry, University of Edinburgh*)

The major changes in P distribution during the incubation of the hen's egg comprise the disappearance of phospholipin and phosphoprotein P and the accumulation of inorganic and (presumably) nucleic acid P (Plimmer & Scott, 1908; Masai & Fukutomi, 1923). These changes, which involve at least 80%, and probably much more, of the total P in the egg, can hardly take place without the intermediate formation of P compounds of low molecular weight. It has therefore been thought worth while to examine changes in the organic acid-soluble P during incubation. Some preliminary results are now presented.

Eggs after known periods of incubation were chilled in acetone-CO_2, stripped of their shells and homogenized while still frozen. The cold suspensions were extracted with cold trichloroacetic acid, and the P in the extracts was fractionated with barium acetate and ethanol at pH 8·3 (modified from LePage, 1948). The method of Allen (1940) proved suitable for the P determinations.

Inorganic P amounts to about 5·2 mg./egg before incubation, rises slowly after the 4th day to 9·8 mg. at 12 days, and then ever more steeply to 50–60 mg. at 21 days. Total organic acid-soluble P starts at about 2·1 mg./egg, rises to a maximum of nearly 11 mg. at 16 days (with a sharp increase from 5 to over 8 mg. between the 10th and 12th days), and then decreases slightly to 9·5–10 mg. at 20–21 days. These figures are broadly similar to those of Masai & Fukutomi.

The three barium-ethanol fractions into which the organic acid-soluble P may be divided behave as follows:

(i) Ba-insoluble organic P remains fairly constant at 0·7–0·9 mg./egg for 8 days, and then rises steadily to 4·5 mg. at 21 days. P hydrolysed in 10 min. at 100° in N-HCl does not account, even in chicks on the point of hatching, for more than about 20% of this fraction (cf. Needham, Nowinski, Dixon & Cook, 1937), but our technique is not proof against partial loss of ATP or ADP.

(ii) Ba-ethanol-insoluble P begins to rise from 0·6 mg./egg at 4 days until it reaches a final value of 4·0 mg. at 21 days. No further investigation has yet been made of this fraction.

(iii) Ba-ethanol-soluble P, which varies over the first 4 days from zero to 1·3 mg./egg, rises to nearly 2 mg. at 10 days, and then to over 4 mg. at 12 days, thus accounting for the sharp jump in total acid-soluble P seen at the same period. After this there is a steady fall until at 21 days the figure is only about 1 mg./egg. Since the quantity of this fraction is not decreased by carrying out the Ba-ethanol precipitation at pH 12 instead of pH 8·3, it does not seen to contain much amino-ethyl phosphate (Outhouse, 1936).

All figures quoted are the means of analyses on 4–10 eggs. Variations from egg to egg were found to be considerable, and only particularly well-defined changes are described here.

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Are Phospholipids Transmitted through the Placenta? By G. Popják and Marie-Louise Beeckmans. (*National Institute for Medical Research, London, N.W. 3*)

It was shown in a previous investigation (Popják, 1947) with the aid of ^{32}P that all foetal tissues in the rat, rabbit and guinea pig are able to carry out the phosphorylating step of phospholipid synthesis. The results strongly suggested that most, if not all, the foetal phospholipids are obtained by synthesis within the foetus. Moreover, studies with deuterium and ^{14}C-labelled acetate (Popják & Beeckmans, 1949 *a*, *b*) proved that even fatty acids are synthesized from smaller molecules within the foetus. The possibility, however, that a small amount of phospholipid, preformed in the mother, might pass through the placenta could not be excluded. The experiments to be reported were designed to probe this question.

The following argument was the basis of the experiments. Two hours after the injection of $^{32}PO_4^{\equiv}$ to a pregnant rabbit, the specific activity of the phospholipid-P in the maternal liver is at the most 5%, and in the foetal tissues (foetal placenta, liver and carcass) 7–15% of the specific activity of the inorganic-P in the corresponding tissues. If after the intravenous injection of labelled phospholipids to pregnant animals the specific activity of the tissue phospholipid-P would exceed the activity of the inorganic-P in any of the organs, this could only mean an active uptake of whole phospholipid molecules from the maternal circulation. Even if the specific activity of the phospholipid-P in the foetal tissues were higher than 15% of the specific activity of the tissue inorganic-P, the result would favour the view that whole phospholipid molecules have passed the placenta.

Three rabbits (18, 23 and 28 days pregnant) were injected intravenously with serum containing phospholipids labelled with ^{32}P. Two hours after the injection the animals were killed and tissue phospholipids and inorganic-$PO_4^{\equiv}$ assayed for ^{32}P.

The results of one experiment are shown in Table 1. Although the foetal placenta took up appreciable amounts of phospholipids from the maternal circulation, it did not transmit whole phospholipid molecules either to the foetal liver or to the rest of the foetus. The radioactivity found in the foetal liver- and carcass-phospholipids was no more than expected from the radioactivity present in the inorganic-P. The results on the 18th and 23rd days of pregnancy were exactly the same, except that the placentae on these dates seem to metabolize phospholipids even more actively than on the 28th day.

Table 1. *Specific activity of phospholipid-P and inorganic-P in maternal liver and in foetus 2 hr. after injection of serum containing* ^{32}P*-labelled phospholipids; 28th day pregnancy*

	Inorganic-P counts/min./mg. (*a*)	Phospholipid-P counts/min./mg. (*b*)	$b/a \times 100$
Maternal liver	184	1009	548
Foetal placenta	1395	2880	207
Foetal liver	823	59	7·2
Foetal carcass*	142	9·4	6·6

* Whole foetus without liver.

The unilateral 'permeability' of the rabbit's placenta to phospholipids is very similar to what was found previously for cholesterol (Popják, 1946), i.e. that the placenta may take up large amounts of cholesterol from the maternal circulation, but does not pass it on to the foetus.

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In Search of a P-precursor of Phospholipids. By G. Popják and Helen Muir. (*National Institute for Medical Research, London, N.W. 3*)

Zilversmit, Entenman & Fishler (1943) showed mathematically that in tracer studies, in which a single injection of the tracer is given to animals, the specific activity-time curves of a substance and its immediate precursor should have a relationship as shown in Fig. 1. The principles laid down by Zilversmit *et al.* have been applied in a search for a specific P-precursor of liver phospholipids (lecithin and cephalin) among the acid-soluble phosphates. Rats were the experimental animals. The fractionation of the acid-soluble phosphates was done as described by Kaplan & Greenberg (1944).

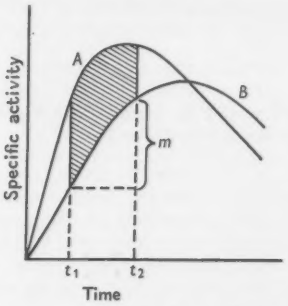

Fig. 1. Relationship between specific activity of precursor (*A*) and product (*B*)

It was found that after a single subcutaneous injection of 10 μc. of ^{32}P as Na_2HPO_4, the only acid-soluble P-fraction, the specific activity-time curve of which fulfilled the criteria for this substance being the specific P-precursor of phospholipids, was a Ba-, Hg- and Pb-soluble, but ethanol-insoluble, organic phosphoric ester. This fraction was identified as consisting mainly of α- and β-glycerophosphate. *In vivo* experiments with both α- and β-glycerophosphate, labelled with ^{32}P, failed to substantiate the suggestion that glycerophosphate is a specific phospholipid precursor. The negative results, however, were well explained by the finding that glycerophosphate does not enter liver cells unhydrolysed. *In vitro* experiments with liver slices, in which ^{32}P-labelled glycerophosphate, glucose-1- and glucose-6-phosphate, muscle adenylic acid and adenosinetriphosphate were used as substrates, similarly gave no evidence that either of these substances might be a specific P-donor to phospholipids because of their rapid hydrolysis by tissue slices.

A short interval after the injection of inorganic PO_4, the specific activities of tissue inorganic-P (I), glycerophosphate-P (II) and of phospholipid-P (III) in descending order are: I > II > III. It was thought, therefore, that if the reaction between phospholipid and its precursor is a reversible one and if this precursor is really glycerophosphate, then, after the intravenous injection of ^{32}P-labelled phospholipids, the specific activities of the P-fractions mentioned above should be in the following order: III > II > I. This argument, of course, should apply only to tissues which take up and metabolize phospholipids. It was found that the rabbit's placenta takes up relatively large amounts of phospholipids from the maternal circulation (see preceding communication), and that 2 hr. after the injection of a serum containing ^{32}P-labelled phospholipids to a 28 days' pregnant rabbit the specific activity of phospholipid-P in the placenta was 2880 c./min./mg., of glycerophosphate-P 2090 c./min./mg. and of the inorganic-P 1395 c./min./mg. It is inferred that the reaction between phospholipid and its precursor is a reversible one and that this precursor is glycerophosphate.

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The Respiration of Rat-Liver Slices in the Presence of some Aliphatic Amines, Hydroxy-amines and Quaternary Ammonium Salts. By C. Long. (*Departments of Physiology and Biological Chemistry, Marischal College, University of Aberdeen*)

Eperjessy & Zathureczky (1947) have stated that the addition of very low concentrations (about 2×10^{-5} M) of choline chloride, tetramethylammonium iodide, or the hydrochlorides of ethylamine, trimethylamine, ethanolamine or triethanolamine markedly lowers the R.Q. of liver slices from fasted rats without affecting the rate of O_2 uptake. They interpret this effect as being caused by a simultaneous stimulation of fat oxidation and inhibition of carbohydrate oxidation by the substances added. Repetition of these experiments has yielded the following results:

(1) Control values for the O_2 uptake by liver slices from fasted rats ($-Q_{O_2}$ range, 5·1–9·0; av. 6·7) were in agreement with data earlier reported in the

literature and nearly twice as high as the figures given by Eperjessy & Zathureczky ($-Q_{O_2}$ range, calc. from their data, 3·1–3·9; av. 3·6).

(2) Control values for R.Q., determined simultaneously with (1) above, gave figures in the range 0·33–0·66, av. 0·50, also in agreement with earlier literature, but quite different from the range 0·92–1·01, av. 0·97, reported by Eperjessy & Zathureczky.

(3) Dimethylamine, trimethylamine, triethylamine, ethanolamine, triethanolamine (as hydrochlorides), choline chloride and tetramethylammonium iodide, when tested at 2×10^{-5} M, had no effect on the $-Q_{O_2}$ or R.Q. values for liver slices from fasted rats. At 2×10^{-2} M, only choline chloride behaved as a substrate, increasing the $-Q_{O_2}$ and reducing the R.Q.

(4) Similar results to those reported under (3) were also obtained with liver slices from well-fed rats.

(5) When tested directly, the substances examined had no effect on (*a*) carbohydrate utilization by liver slices from well-fed animals or (*b*) glyconeogenesis in liver slices from fasted animals.

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Paper Chromatography of the Vitamin B_{12} Group of Factors. By E. Lester Smith and W. F. J. Cuthbertson. (*Research Division, Glaxo Laboratories Ltd., Greenford, Middlesex*)

In January 1949 we reported the separation of four active factors from liver extracts and from bacteria by a combination of paper-strip chromatography and microbiological assay on a solid medium. Three were characterized as 'slow-moving A.P.A.F.', 'crystalline A.P.A.F.' (now known to be identical with vitamin B_{12}) and thymidine. It is now clear (see references) that all five naturally occurring purine and pyrimidine deoxyribosides are microbiologically active, and Winsten & Eigen (1949) showed the presence in liver extracts of six (unidentified) growth factors for *L. leichmanii* 313.

ellipticity of the zones of growth on agar, and comparing their maximum diameters with those of standard (undeveloped) rings. The results were independent of the load from 0·003 to 0·8 μg. However, crude products often show imperfect separation of the two zones. In other instances the slow-moving factor has clearly split into two.

Some deoxyribosides are difficult to separate chromatographically, especially those of guanine and hypoxanthine, and in some solvents those of adenine and thymine. We have now chromatographic evidence for the presence in extracts from liver and

R_F values in various solvents

Substance	Methyl isobutyl ketone	Methyl ethyl ketone*	Secondary butanol†	*n*-Butanol + 10% acetic acid	*n*-Butanol	Winsten & Eigen *n*-butanol	
Slow-moving A.P.A.F.	0	0	0·04–0·09	0·10–0·14	0·03–0·08	1	0–0·03
Vitamin B_{12}	0	Negligible	0·18–0·28	0·25–0·30	0·06–0·13	2	0·03–0·1
						3	0·15–0·2
Cytosine deoxyriboside	—	0·09–0·11	—	0·35–0·37	0·28–0·34		
Guanine ,,	—	0·12–0·14	0·44–0·55	*c.* 0·40	*c.* 0·35	4	0·26–0·36
Hypoxanthine ,,	*c.* 0·01	0·13–0·16	0·44–0·55	0·36–0·46	0·32–0·36		
Adenine ,,	0·02–0·025	0·28–0·35	0·61–0·69	0·46–0·61	0·51–0·57	5	0·41–0·54
Thymine ,,	0·06–0·065	0·44–0·47	0·71–0·76	0·62–0·73	0·57–0·60	6	0·52–0·67

* 90% saturated with water; developed in cold room. Other solvents saturated with water; developed at room temperature.
† Tank not humidified.

We have extended our work in several directions. Some factors can seriously upset R_F values unless controlled. Volatile (water-saturated) solvents tend to evaporate, leaving the paper strip excessively moist, while water evaporates preferentially from other solvents like butanol.

Secondary butanol was suitable for separating vitamin B_{12} from slow-moving A.P.A.F. The proportions could be assayed roughly by ignoring the

from *Streptomyces griseus*, of the deoxyribosides of thymine, adenine, cytosine and either hypoxanthine or guanine; the latter correspond with the 'Band 3' we reported previously. The probable identity of five of Winsten & Eigen's six factors is indicated in the table.

The plate assay of liver extracts for vitamin B_{12} can be made more accurate by removal of the interfering deoxyribosides; this can be done by

measuring microdrops of the test and standard solutions on to a sheet of filter paper and carrying out ascending development with wet *n*-butanol for an hour or two; the paper is then cut away beyond the zone of riboflavin used as marker; the resulting zones of growth have sharp edges and are only slightly elliptical.

We wish to thank Dr T. Brady, Dr T. Jukes and Dr V. Kocher for generous gifts of various deoxyribosides.

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Irreversibility of the Deamination of Threonine in the Rat. By D. F. Elliott and A. Neuberger. (*National Institute for Medical Research, Hampstead, N.W.* 3)

Feeding experiments with amino-acids labelled with ^{15}N in the α-position have shown that most of them take part in reversible deamination reactions in the animal body. This is true whether the amino-acids are essential for growth or not. Weissman & Schoenheimer (1941) found, however, that lysine did not exchange its α-nitrogen atom for that supplied by other amino-acids. This is in keeping with the observation of Berg (1936) that D-lysine is ineffective for growth purposes. The case of threonine, in which the D-isomer is known to be ineffective for growth (Rose, 1938), has not hitherto been investigated.

Three adult rats, which had been kept for a preliminary period on a low-protein diet, were given for three successive days isotopic glycine (150 mg./day/rat) containing 31·5 atom % excess ^{15}N. At the end of the fourth day the animals were killed and the proteins of the internal organs pooled and hydrolysed. Serine and threonine were isolated from the hydrolysate by the method recently described by one of us (Elliott, 1949); various other amino-acids were isolated by well-known methods. The results of the isotope analyses, given in Table 1, clearly show that threonine does not take part in reversible deamination reactions to any detectable extent under the conditions of the experiment. We are indebted to Dr R. Bentley for the mass spectrometric analyses.

Table 1. *Atom % excess of* ^{15}N *in six amino-acids isolated from the internal organs of three rats which had been fed glycine containing* 31·5 *atom % excess of* ^{15}N

Amino-acid	Atom % excess ^{15}N
Glycine	3·500
Serine	3·300
Threonine	0·000
Arginine monohydrochloride	0·366
Tyrosine	0·107
Lysine monohydrochloride	0·000

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Paper-Partition Chromatography of the Sugars in Urine. By Marjory Eastham (introduced by R. P. Cook). (*Department of Biochemistry, University College, Dundee*)

The carbohydrates present in samples of urine have been studied by paper-partition chromatography (Partridge, 1948) using as partition solvent normal butanol 4, acetic acid 1, water 5.

With untreated urines a white mineral trail develops after treatment with ammoniacal $AgNO_3$ and obscures a number of sugars, but if the urine is treated with basic lead acetate, concentrated and extracted with methanol, the extract consists mainly of carbohydrates and gives good chromatograms.

Normal and diabetic urines and the urine from one case of infective hepatitis and the urine from a patient on caronamide treatment have been studied. Variable amounts of glucose are present in all urines. A reducing material of R_F value 0·37 is present in large amounts. This compound has not

been identified, but the methyl pentose rhamnose gives a similar value and chemical reactions. 'Spots' are present at positions suggesting arabinose, xylose and fructose. Uronic acids (including ascorbic acid) are apparently present in all the urines investigated. A number of reducing 'fast runners' are also found. These have not been identified.

The urine of the patient on caronamide treatment showed a great increase in the amounts of pentoses and uronic acids.

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The Distribution of Lipoids in the Faeces of Normal and Cholesterol-fed Animals. By R. O. Thomson. (*Department of Biochemistry, University College, Dundee*)

The distribution of lipoids in the faeces of rats, guinea pigs and rabbits, fed a diet consisting of rat-cake (North Eastern Agricultural Co-operative Society, Aberdeen), plus olive oil, with and without cholesterol, has been investigated.

Table 1. *Controls* (*as percentage total lipoid*)

Fraction	Rats	Guinea pigs	Rabbits
Unsaponifiable matter	23·5	12·0	19·0
Total sterol	8·5	2·2	4·9
Ester sterol	3·5	0·4	1·9
Unsaponifiable matter not sterol	16·0	9·9	14·1
Total fatty acids	75·7	75·2	61·8
Free acids: (*a*) Calculated	30·1	79·6	49·6
(*b*) Actual wt.	20·3	71·6	21·0
Ester sterol fatty acids	2·6	0·3	1·4
Glyceride acids	52·8	3·3	39·4

The distribution of the main constituents for the control animals is shown in Table 1.

The values for the cholesterol-fed animals are given in Table 2.

Table 2. *Cholesterol-fed* (*as percentage total lipoid*)

Fraction	Rats	Guinea pigs	Rabbits
Unsaponifiable matter	42·6	26·7	47·1
Total sterol	27·4	13·3	18·8
Ester sterol	8·5	1·6	3·7
Unsaponifiable matter not sterol	13·9	13·4	28·3
Total fatty acids	32·5	63·2	39·0
Free acids: (*a*) Calculated	25·8	61·6	28·5
(*b*) Actual wt.	7·8	59·6	6·7
Ester sterol fatty acids	4·2	1·2	2·7
Glyceride acids	20·5	2·4	29·5

The volatile acids present have been estimated and separated by partition chromatography.

Some Observations on the Structures of Certain Fructosans. By D. J. Bell and Anne Palmer. (*Biochemical Laboratory, University of Cambridge*)

The following preliminary results are reported.

A. *Inulins.* Specimens (several times recrystallized from water) were hydrolysed and estimations of D-glucose carried out, using the specific D-glucose oxidase from *Penicillium notatum* (Keilin & Hartree, 1948). Inulin from *Dahlia* was fractionated into rapidly and slowly crystallizing material; inulin from *Taraxacum* and from *Inula* were not fractionated.

The following results were obtained:

Source	D-Glucose moles %	Chain-length per mole glucose
Dahlia: Rapidly crystallized	2·5	38
Slowly crystallized	2·2	43
Taraxacum	2·5	37
Inula	2·3	38
Control (D-fructose treated under same hydrolytic conditions)	0·0015	—

The above results of 'chain length' are of the same order as those found by Haworth, Hirst & Percival (1932) by normal end-group assay assuming no glucose to be present. It must be noted that Irvine & Montgomery (1933) showed that hydrolysates of methylated inulin contained some *trimethyl* D-glucose. It is therefore not clear whether inulin contains glucose as a terminal or an intermediary radical.

B. *Grass levan.* Using the partition chromatogram of Bell & Palmer (1949) end-group assay results are similar to those obtained by others (vide supra). However, an appreciable amount of dimethyl fructose was found among the hydrolysis products. Therefore the levans are either branched polysaccharides or the chains are strongly hydrogen-bonded one with another, thus resisting etherification.

Source (grass)	1:3:4:6-Me_4 fraction (moles)	1:3:4-Me_3 fraction (moles)	Me_2 fraction (moles)	'Chain length'
Italian rye	1	12	1	14
Leafy cocksfoot	1	11	1·7	14

Dr A. G. Ogston of Oxford has determined (by sedimentation and diffusion constants) the molecular weight of the leafy cocksfoot material. This is of the order of 5,200–5,400. Perennial rye-grass levan has a similar molecular weight. It is clear that grass levans are not simple chain molecules. We have further shown that bacterial levans are larger in size, since they do not dialyse through cellophane while grass levans are freely diffusible.

C. *Irisin*. Using partition and paper chromatography we have shown that methylated irisin contains an appreciable fraction of trimethyl fructoses (missed by previous workers). This consists of about 2 parts of 1:3:4-trimethyl D-fructose with 1 part of the (probably) 3:4:6-trimethyl isomer. The results of our experiments are detailed below. (The dimethyl fructose fraction has been shown to be the 3:4-isomer.)

1:3:4:6-Me_4 fructose (moles)	1:3:4-Me_3 fructose (moles)	(?)3:4:6Me_3 fructose (moles)	3:4-Me_2 fructose (moles)	'Chain length'
7	2	1	7	17

Dr Ogston has determined the mean molecular weight of our irisin as 22,500. This indicates that our material contains 8–9 unit chains.

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Observations on the Renal Clearance of some Fructosans. By J. Beattie and A. C. Corcoran (introduced by D. J. Bell). (*Bernhard Baron Research Laboratories, Royal College of Surgeons of England, London and Cleveland Clinic, Cleveland, Ohio, U.S.A.*)

The renal clearances of levan (from Italian rye-grass) and irisin (from iris rhizomes) were determined in unanaesthetized dogs by using either the single dose technique or continuous infusion over a period of 2–3 hr. Simultaneous determinations of creatinine, mannitol and sodium thiosulphate clearances were made. The ratio of fructosan to creatinine clearance declined as shown in the table.

	1st sample (15 min.)	End of 1st hour	End of 2nd hour
Levan:			
Single dose	1·0	0·35	0·2–0·3
Continuous infusion	0·72	0·48	0·4–0·45
Irisin:			
Single dose	0·85	0·62	0·3
Continuous infusion	0·60	0·45	0·4–0·5

The results suggested that neither of the fructosans was homogeneous. The fall in the absolute fructosan clearance and that relative to creatinine or thiosulphate clearance were independent of plasma level. Histamine in doses sufficient to cause renal vasodilation produced no change in the absolute or relative fructosan clearance.

The Changing Chemical Composition of Chick-Heart Explants Grown *in vitro*. By J. N. Davidson and I. Leslie. (*Department of Biochemistry, Glasgow University*)

By modifying the roller-tube technique employed in earlier work on chick-heart explants *in vitro* (Davidson, Leslie & Waymouth, 1949), it has become possible to determine the changing amounts of a number of P and N components of the growing cultures. In addition to the measurement of the acid-soluble P and lipid P, the ribonucleic acid phosphorus (RNAP) and deoxyribonucleic acid phosphorus (DNAP) fractions are determined by a modified form of the Schmidt & Thannhauser (1945) method, while N is determined by the micro-Kjeldahl method (Markham, 1942).

Evidence from other sources is accumulating to show that the DNA content of the nuclei of all somatic cells is constant in amount for each particular species, excluding, perhaps, those of certain mammalian organs (Vendrely & Vendrely, 1948; Mirsky & Ris, 1949). On this assumption the amounts in DNAP can be taken as directly proportional to the number of cells in the cultures at various stages of growth. The approximately sevenfold increase in DNAP recorded during 7 days' growth implies a similar increase in the number of cells. Most of this increase occurs after the third day of growth.

The chemical composition of the explants during growth changes in a regular fashion, and when expressed as the number of P and N atoms of the various fractions relative to atoms of DNAP, the results can be interpreted as the changes in composition of the cells. The general trend is a decrease

in the amounts of protein N, acid-soluble P, lipid P, and RNAP relative to the DNAP, particularly after 3 or 4 days of growth. The relative proportions of these components, however, show little change in the later stages of growth, when the DNAP is still increasing appreciably in the cultures as a whole.

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Phosphorus Compounds in the Cell Nucleus. By J. N. Davidson and W. M. McIndoe. (*Biochemistry Department, Glasgow University*)

Isolated cell nuclei from rabbit liver, fowl erythrocytes and calf thymus have been submitted to the fractionation procedures of Schmidt & Thannhauser (1945) and of Schneider (1945) and to a combination of these methods, neither of which is, by itself, altogether satisfactory. The phosphorus content of each fraction was estimated. The results for the nucleic acid fractions have been compared with figures from pentose and deoxypentose estimations by a number of different methods.

Cell nuclei contain acid-soluble phosphate esters, phospholipids (relatively abundant in erythrocyte nuclei) deoxyribonucleic acid phosphorus (DNAP) and small amounts of ribonucleic acid phosphorus (RNAP), phosphoprotein phosphorus (PP), protein-bound phosphorus insoluble in acid and warm N-NaOH (IP) and a protein-bound phosphate ester (EP).

With the aid of ^{32}P these compounds have been shown to be biologically distinct. When the various phosphorus fractions are separated from nuclei obtained from animals which have received radiophosphorus, the phospholipid has a low specific activity, the DNAP is found to have a much lower specific activity than the RNAP, the EP has an activity of intermediate value, while IP and PP have very high activities, comparable with those for acid-soluble phosphorus. High activity for phosphoprotein phosphorus in whole tissues has already been recorded (Davidson, Gardner, Hutchison, McIndoe, Raymond & Shaw, 1949).

The amount of nucleic acid in 10^{-6} μg./nucleus has been found to be as follows:

	DNA	RNA
Fowl erythrocyte nuclei	2·62	0·09
Rabbit-liver nuclei	7·23	0·70

This is in agreement with the results of Vendrely & Vendrely (1948) and of Mirsky & Ris (1949).

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Stercobilin Formation in a Case of Congenital Porphyria and in the Normal. By C. H. Gray, A. Neuberger and P. H. A. Sneath

The investigations with glycine labelled with ^{15}N which have been reported (Gray & Neuberger, 1949) have been further extended, especially by carrying out a control experiment on a normal male. The ^{15}N content of the haemin in the porphyric rises to a much greater value than in the normal. The maximum, which is reached after about 12 days, is followed by a marked drop. The later part of the curve appears to be normal, indicating that the majority of the red cells have a life span of about 120 days.

It will be observed from Table 1 that even in the normal the stercobilin excreted during the first few days of the experiment contains a fairly high concentration of ^{15}N. This finding, which necessarily implies that not all the stercobilin can be derived from the breakdown of red cells of a normal life span, might be explained by assuming that a very small number of cells die before they reach the circulation, or have an exceedingly short life span, what might be termed an 'infantile mortality'. It is also possible that a fraction of the stercobilin arises

from the breakdown of haem proteins other than haemoglobin, which may have a rapid turnover rate in the body, or from intermediary porphyrins or their precursors. London, West, Shemin & Rittenberg (1948) have also found a high ^{15}N content of stercobilin in the early period in similar experiments.

Table 1. *Atom percentage excess ^{15}N of stercobilin in normal male*

Days after feeding glycine	Atom % excess ^{15}N
1–4	0·085
5–8	0·161
9–12	0·067
13–16	0·068
29–32	0·018
57–60	0·018

The results obtained from the porphyric (Table 2) suggest that in this condition a certain proportion of the red cells have an exceedingly short life span. Such an assumption would explain the relatively high ^{15}N content of the haemin and of the stercobilin soon after the beginning of the experiment.

There are highly significant amounts of ^{15}N in the stercobilin of the 30th to the 80th days in both cases, but more in the porphyric. It is not yet clear whether this is due to the breakdown of a very small number of the labelled red cells, or whether it represents stercobilin derived from a second unknown source. During the period when the majority of labelled red cells are being destroyed, the isotope content of the stercobilin in the case of porphyria rises. The rise is much less than would be expected from the fall in the haem ^{15}N, and confirms the conclusion that only a small fraction of the stercobilin is derived from the breakdown of red cells of a normal life span. The stercobilin from the case of congenital porphyria has been shown to be identical with that of normal faeces by elemental analysis, optical rotation and spectral absorption curves.

Table 2. *Atom percentage excess ^{15}N of stercobilin in congenital porphyria*

The ^{15}N content of stercobilin during the first 28 days has already been reported (Gray & Neuberger, 1949).

Days after feeding glycine	Atom % excess ^{15}N
33–37	0·108
38–42	0·089
43–46	0·080
81–86	0·064
87–92	0·064
93–99	0·072
100–105	0·070
106–112	0·064
119–124	0·072
125–131	0·088
132–139	0·091

We wish to thank Dr R. Bentley for determining the isotope concentrations.

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DEMONSTRATIONS

Cup Plate Assay of Vitamin B_{12}. By W. F. J. Cuthbertson and Joan T. Lloyd

Potency Calculator for Use with Logarithmic Dose Response Curves. By B. Basil

The Chemical Changes in Carbohydrates During Growth of the Pea (*Pisum sativum*). By Marjory Eastham (introduced by R. P. Cook). (*Department of Biochemistry, University College, Dundee*)

Sections of the pea during various stages of growth have been stained using histochemical reagents to demonstrate pectin, starch, cellulose and lignin development.

The soluble sugars have been studied by paper-partition chromatography.

Chemical Constituents of the Pea (*Pisum sativum*). By R. P. Cook and Margaret B. Brown. (*Department of Biochemistry, University College, Dundee*)

FORTHCOMING PAPERS

It is hoped to publish the following papers in the next issue of the *Biochemical Journal*:

The distribution of glycogen in the liver of rabbits. By A. Nys, X. Aubert and C. de Duve

Prosthetic groups of the cytochromes present in *Corynebacterium diphtheriae* with especial reference to cytochrome *a*. By W. A. Rawlinson and J. H. Hale

The oxidation of manganese by plant extracts in the presence of hydrogen peroxide. By R. H. Kenten and P. T. G. Mann

The biochemistry of locusts. I. The carotenoids of the integument of two locust species (*Locusta migratoria migratorioides* R. & F. and *Schistocerca gregaria* Forsk.). By T. W. Goodwin and S. Srisukh

Some observations on astaxanthin distribution in marine crustacea. By T. W. Goodwin and S. Srisukh

The intermediary metabolism of the mammary gland. 2. Respiration and acid production of mammary tissue during pregnancy, lactation and involution in the rat. By S. J. Folley and T. H. French

Tryptophan and the biosynthesis of nicotinamide. By P. Ellinger and M. M. Abdel Kader

Some observations on the amino-acid distribution of collagen, elastin and reticular tissue from different sources. By J. H. Bowes and R. H. Kenten

A differential method for the detection of small differences in mobility of colloids in electrophoresis. By H. Hoch

Chromatographic studies on nucleic acids. 1. A technique for the identification and estimation of purine and pyrimidine bases, nucleosides and related substances. By R. Markham and J. D. Smith

Studies in vitamin A:

10. Vitamin A_1 and retinene$_1$ in relation to photopic vision. By S. Ball and R. A. Morton

11. Reactions of retinene$_1$ with amino compounds. By S. Ball, F. D. Collins, P. D. Dalvi and R. A. Morton

12. Whale-liver oil analysis: preparation of kitol esters. By R. H. Barua and R. A. Morton

13. The alleged formation of vitamin A from β-carotene treated with iodinated casein. By H. R. Cama and T. W. Goodwin

The pyruvate-oxidase system in brain and the tricarboxylic acid cycle. By R. V. Coxon, C. Liebecq and R. A. Peters

Acceleration of bacterial glutamic decarboxylase and glutaminase by cetrylmethylammonium bromide. By D. E. Hughes

Factors influencing the polysaccharide content of *Escherichia coli*. By S. Dagley and E. A. Dawes

Breakdown of the oxidized forms of coenzymes I and II by an enzyme from the central nervous system. By H. McIlwain and R. Rodnight

Apparent vitamin C in the walnut (*Juglans regia*). By F. Wokes and R. Melville

Chemical factors in the germination of spore-bearing aerobes.

1. The effect of yeast extract on the germination of *Bacillus anthracis* and its replacement by adenosine. By G. M. Hills

2. The effects of amino-acids on the germination of *Bacillus anthracis*, with some observations on the relation of optical form to biological activity. By G. M. Hills

Studies in vitamin A. 14. The alleged mobilization of vitamin A by adrenalin. By T. W. Goodwin and A. A. Wilson

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the author.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **279th Meeting** *of the Biochemical Society was held in the Department of Biochemistry, School of Medicine, Leeds* 2, *on Friday,* 23 *September* 1949, *when the following papers were read:*

COMMUNICATIONS

The Effect of Thyroxine and Thiouracil on some of the Water-soluble Vitamins in Milk. By R. CHANDA, MARY L. MCNAUGHT and E. C. OWEN. (*Hannah Dairy Research Institute, Kirkhill, Ayr*)

In a previous communication Chanda & Owen (1949) showed that thyroxine markedly affected the partition of P in cows' milk, and that the changes in the partition were correlated with simultaneous changes in the phosphatase content of the milk. They also showed that milk from cows treated with thiouracil exhibited changes of phosphatase content and P partition which were the reverse of those caused by thyroxine. In the same experiments thyroxine treatment caused the vitamin C in the milk to decrease by 26·3% and thiouracil caused it to increase by 22·2%, the average of pre-treatment figures for this vitamin in the milk of treated cows being 2·23 mg./100 ml. Houston, Kon & Thompson (1940) had found an inverse correlation between vitamin B_1 pyrophosphate and phosphatase in the milk of normal cows, and experiments were therefore planned to find whether thyroxine or thiouracil would alter the amount of B_1-phosphoric esters in the milk. The term 'B_1-phosphoric esters' is preferable to 'B_1-pyrophosphate', since the nature of the B_1-esters in milk of various species is still controversial, both pyrophosphate and orthophosphate being present (de Jong, 1941–2).

In the present experiment three pairs of cows were used. One pair acted as controls. A second pair received 10 mg. thyroxine per day subcutaneously, while a third pair received 20 mg. thiouracil subcutaneously. Composite 2-day samples of milk from each cow were analysed for 3 weeks before treatment (period 1), for 3 weeks during treatment (period 2), and for 3 weeks after treatment (period 3). The average results for each period (Table 1) show that, like ester-P in the previous experiment (Chanda & Owen, 1949), phosphoric esters of B_1 increased as phosphatase decreased when thyroxine was given, while when thiouracil was given the reverse occurred. The disturbance of the ratio of free to phosphorylated vitamin B_1 by thyroxine confirms the similar observation by Thompson (1945). Total riboflavin did not vary significantly during treatment, and, unfortunately, the partition of riboflavin into free and phosphorylated forms could not be investigated owing to lack of a method adaptable to routine analysis.

Table 1. *The riboflavin content and partition of vitamin B in milk*

			(μg./100 ml. fat-free milk)				
Group of animals	Period	Phosphatase (arbitrary units)	Total B_1	Free B_1	B_1-phosphoric esters	Protein bound B_1	Riboflavin
Control	1	53	42·9	21·1	16·8	3·15	92·9
	2	62	39·3	23·7	10·9	2·79	91·3
	3	83	38·8	27·6	6·4	2·59	89·6
Thyroxine	1	68	44·3	27·0	12·9	2·73	107·3
	2	28	43·5	12·9	25·4	3·80	96·3
	3	78	40·2	23·8	11·2	2·63	104·2
Thiouracil	1	38	48·1	21·2	21·1	3·57	115·9
	2	67	42·1	28·4	9·5	2·69	109·6
	3	66	41·0	27·4	8·5	2·77	103·3

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Some Remarks on the Hydrolysis of Proteins. By H. M. Flowers and W. S. Reith (Reich).

The Energy of Activation of the Tryptophanase-tryptophan Indole Reaction. By Frank C. Happold and G. A. Morrison. (*Department of Biochemistry, School of Medicine, University of Leeds*)

The energy of activation has been studied in the range of temperatures from 0 to 37° C. The provisional figure of 18,200 cal./g.mol. is given.

Absorption and Excretion of DL-Thyroxine Labelled with Radioactive Iodine. By J. C. Clayton, Audrey A. Free, J. E. Page, G. F. Somers and E. A. Woollett. (*Research Division, Glaxo Laboratories Ltd., Greenford, Middlesex*)

DL-Thyroxine labelled with ^{131}I in the 3′ and 5′ positions has been prepared by an improved procedure (Borrows, Clayton & Hems, 1949), and its absorption and excretion by rats and cats have been studied.

Joliot, Courrier, Horeau & Süe (1944*a*,*b*), Joliot (1945) and Gross & Leblond (1947) have investigated the absorption and excretion of relatively large intravenous doses of labelled DL-thyroxine by rabbits and rats. In view of the doubts about the efficacy of orally administered thyroxine, we were particularly interested in its absorption from the digestive tract. We have also studied the behaviour of physiological doses by the intravenous and subcutaneous routes.

The labelled thyroxine solutions were shown to be biologically active (Smith, Emmens & Parkes, 1947) and all preparations were polarographically assayed for thyroxine (Borrows, Hems & Page, 1949). The urine, blood, bile, faeces and organs from the treated animals were digested and the radioactivity of an aliquot portion was measured in a jacketed Geiger-Müller counter (Veall, 1948). The amounts of ^{131}I present in the bile, urine and faeces, as thyroxine, diiodotyrosine and iodide, were measured by Gross and Leblond's method (1947).

Experiments on rats that had received oral doses of 1·0 mg. of labelled thyroxine per kg. of body weight showed that thyroxine was absorbed from the gut, and that 95% of the dose was excreted during the first four days. The ^{131}I in the urine was present almost entirely as iodide, whereas that in the faeces was present in both organic and inorganic forms. Our results were in general agreement with those of Gross & Leblond (1947).

Anaesthetized cats were used to study the amount of ^{131}I in blood and its excretion in the bile and urine. The cats received single doses of 34–100μg. of labelled thyroxine per kg. of body weight, subcutaneously, intravenously or by direct injection into the duodenal lumen. Absorption occurred from the intestinal lumen. For all routes, the amount of ^{131}I in the bile was related to the blood concentrations; the ^{131}I in the bile was present in approximately equal proportions in organic and inorganic forms. Appreciable amounts of ^{131}I were secreted into the stomach, but very little activity was found in the thyroid glands. About 6% of an intra-duodenal dose was excreted in the urine during the first 6 hr. after injection.

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Some Observations on the Co-enzyme of Phosphoglucomutase. By L. H. STICKLAND

Trucco, Cardini, Paladini, Caputto & Leloir (1948) have recently shown that glucose-1:6-diphosphate (GDP) is the co-enzyme of yeast phosphoglucomutase (PGM), and suggest that the claim of Kendal & Stickland (1938) that fructofuranose-1:6-diphosphate (FFDP) is the co-enzyme was due to contamination of their FFDP with a trace of GDP. This communication aims at showing that, although Kendal and Stickland's FFDP was indeed contaminated with GDP, yet FFDP can take the place of the co-enzyme of muscle PGM.

The evidence is:

(1) The apparent affinity of yeast PGM is much smaller than that of muscle PGM for crude FFDP. According to Sutherland, Posternak & Cori (1949) the two enzymes have the same affinity for GDP.

(2) FFDP purified by repeated crystallization of the brucine salt is inactive to yeast PGM, but still active to muscle PGM. Roughly one-half of the activity of the crude FFDP is due to its GDP content, which is of the order of 1%.

(3) During acid hydrolysis of pure FFDP, co-enzyme activity is lost *pari passu* with the hydrolysis of the first phosphoric acid. GDP, on the other hand, is completely inactivated by very mild acid hydrolysis (Trucco *et al.* 1948).

(4) On alkaline hydrolysis of pure FFDP, removal of 85% of the phosphoric acid destroys the activity to muscle PGM, while similar treatment of the crude product leaves the activity to the yeast enzyme unchanged.

(5) Partial hydrolysis of the crude FFDP gives results consistent with the view that the activity is due one half to GDP and one half to FFDP.

FFDP obviously cannot participate in the chain of reactions suggested by Trucco *et al.* as the mechanism of the action of GDP. It seems much more likely that FFDP acts by transferring a phosphoric acid to glucose-1-phosphate, to produce GDP, which is the true co-enzyme. All the present experiments were done with a partially purified muscle PGM; the behaviour of a highly purified enzyme (Jagannathan & Murray Luck, 1949) is still to be studied.

Another point of difference between the enzymes from yeast and from muscle is that, whereas yeast PGM in the absence of GDP is completely inactive, partly purified muscle PGM is not. After a lag period the reaction begins slowly, gathers speed, and eventually reaches a rate equal to that found at optimal co-enzyme concentration. This may possibly account for the fact that several workers have purified muscle PGM without observing that it needs a co-enzyme.

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Amino-acid Oxidase of *Neurospora crassa*. By A. E. BENDER, H. A. KREBS (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Sheffield*) and N. H. HOROWITZ (*Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California*)

Horowitz (1944) reported that aqueous extracts of *Neurospora crassa* contained an amino-acid oxidase capable of converting D-α-amino-acids into the corresponding α-ketonic acids and ammonia. The enzyme was found to be inactive against L-amino-acids, except L-glutamic acid which was oxidized at a rate less than one-fifth of that found for the D-compound.

A strain of *N. crassa* obtained from the National Type Culture Collection (no. 3411) was found by Bender & Krebs to produce a highly active amino-acid oxidase, but only L-amino-acids were attacked by this enzyme. A second strain of *N. crassa*, which was obtained from Mr G. H. Wiltshire, Cambridge, also produced an L-amino-acid oxidase.

The apparent discrepancy between these results and those reported by Horowitz were studied jointly at the Sheffield and Pasadena laboratories and was found to be due to strain differences. Under identical conditions some strains form a D-amino-acid oxidase only and some an L-amino-acid oxidase only. Other strains form both enzymes.

The formation of the two enzymes also depends on the composition of the medium. Both enzymes are formed (by suitable strains) when the synthetic medium of Horowitz & Beadle (1943) with 0·25 μg.

biotin per litre is used, but increasing the biotin concentration to 5 μg./l. suppresses the production of the L-enzyme by the Cambridge strain.

The L-amino oxidase was present not only in extracts of the mycelium but also in the clear liquid culture medium. In some experiments over 80% of the total enzyme produced in the culture flask was present in the medium after 15–18 days of incubation. The D-amino-acid oxidase is not discharged into the medium.

Knight (1948) has recently described an L-amino-acid oxidase in acetone-dried mycelia of several *Penicillium* species and of *Aspergillus niger*. This enzyme seems to differ in several ways from that found in *Neurospora crassa*. The quantity of L-enzyme produced by *N. crassa* can be of the order of 100 times greater, on a weight-for-weight basis, than that produced by the other moulds. There are also differences in the relative rates at which different amino-acids are attacked. The *Neurospora* enzyme, unlike Knight's, is not inhibited by narcotics such as octanol.

The L-amino-acid oxidase is the second oxidase found to be released by a mould into the medium, the first case being that of glucose oxidase (Coulthard, Michaelis, Short, Sykes, Skrimshire, Standfast, Birkinshaw & Raistrick 1945). Together with L-amino oxidase, *N. crassa* releases a highly active catalase into the medium. Hence no accumulation of hydrogen peroxide is expected when the oxidase acts upon amino-acids under normal growth conditions.

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Plasma and Tissue Protein Regeneration in Renal Disease with Hypoproteinaemia and Oedema.

By FREDA K. HERBERT. (*Section of Chemical Pathology, Department of Pathology, King's College, Newcastle-upon-Tyne*)

A few studies hitherto reported show that in this type of renal disease the administration of a diet high in both protein and total calories causes positive N balance which may be maintained for many months, though restoration of plasma protein may be very slow (Peters, Bruckman, Eisenman, Hald & Wakeman, 1931; Liu & Chu, 1935; Keutmann & Bassett, 1935; Farr, 1938; Cowie, Jarvis & Cooperstock, 1930). The object of the present work was to determine whether cystine, given as a supplement to diets of a type expected to give the highest N balance so far obtainable, would increase the rate of plasma protein regeneration. Heavy albuminuria involves loss of serum albumin, which is richer in cystine than any dietary protein. Some observations of Whipple and his collaborators suggested that cystine might have a specific effect on plasma protein regeneration (Madden, Noehren, Waraich & Whipple, 1939; Robscheit-Robbins, Miller & Whipple, 1943).

Four patients with nephritis, giving a history of insidious onset of oedema without gross haematuria, have been studied. Total serum protein ranged from 3·27 to 4·11%; albumin from 1·37 to 2·15%. Data were obtained for total N in diet, urine, and faeces; fluctuations in N.P.N. of body fluid; serum

Case	1		2		3		4	
Sex	F.		M.		M.		M.	
Age	27		40		33		17	
Duration of illness	7 months		3 months		17 months		10 months	
Cystine given, g./day	0	0·5	0	2·7	0	2·7	0	2·4
Protein (g. N/day)								
(*a*) Absorbed	7·56	7·57	17·33	17·28	17·29	17·39	16·93	17·17
(*b*) Catabolized	3·35	5·11	10·95	11·41	11·08	10·15	12·29	10·84
(*c*) Gained in tissues	2·23	0·87	3·89	2·74	3·38	4·48	1·32	3·34
(*d*) Gained or lost in plasma	+0·19	−0·07	0·00	−0·14	+0·27	−0·14	+0·36	+0·04
(*e*) Lost as urinary protein: Albumin	1·79	1·66	2·17	2·79	2·20	2·47	2·29	2·13
(*e*) Lost as urinary protein: Globulin			0·31	0·48	0·36	0·43	0·67	0·82
(*f*) Total protein synthesized ($c+d+e$)	4·21	2·46	6·37	5·87	6·21	7·24	4·64	6·33

proteins, plasma volume, and urinary protein. Total calorie intakes ranged from 2613 to 3412 per day. Protein intake ranged from 116 to 124 g. per day except in case 1, in which there was a low urea clearance. Case 2 was complicated by recurrent infection, and very slight pyrexia was present during the balance study. The diet was given for 4–9 days in preparation, for the next 7–10 days as a balance period, and for a further 7–10 days' balance period with added cystine. The dose of cystine was planned to bring the cystine:lysine ratio of the dietary protein up to that of serum albumin. The essential findings are given in the table.

On diet alone, there were high positive N balances due to tissue protein regeneration. A high rate of serum protein regeneration was offset by albuminuria. In no case did the cystine improve the rate of serum protein regeneration appreciably. In case 3, cystine appeared to produce a definite improvement in tissue protein regeneration. In case 4, a growing boy, a moderate rate of tissue protein regeneration on diet alone was greatly increased by the cystine supplement.

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Transport of Radioactive Na^+ and K^+ Through Gastric Mucosa. By E. Eva Crane and R. E. Davies. (*Unit for Research in Cell Metabolism* (*Medical Research Council*), *Department of Biochemistry, University of Sheffield*)

Experiments with radioactive ions to discover the flux of Na^+ and K^+ across gastric mucosa under a variety of conditions have been carried out as part of an investigation into the secretory and electrical activity of this membrane. Isolated frog gastric mucosa was mounted in the apparatus described by Crane, Davies & Longmuir (1948). The nutrient solution was frog bicarbonate saline; the secretory solution was either 0·12 M-NaCl or 0·12 M-KCl. The solution bathing one side contained a known concentration of ^{24}Na or ^{42}K; the solution bathing the other side was changed periodically and its radioactivity measured with a liquid counter.

A complication arose when it was discovered that even when the radioactive isotope (in a solution of total molarity 0·12) was diluted 250-fold with water, losses of activity up to 30% occurred due to exchange of radioactive ions with ions in the walls of glass vessels, syringes, etc. The activity could be recovered by washing with solutions of the stable isotope, and the loss of activity did not occur when dilutions were made with 0·12M-NaCl or 0·12M-KCl.

For any one resting mucosa the rate of transport of Na^+ or K^+ remained constant to $\pm 15\%$, but increased rapidly if the mucosa was mechanically damaged as indicated by the fall of the potential difference across it. Experimental results given in Table 1 are based on averages for at least three consecutive half-hour periods. They show that the rates of transport of Na^+ and K^+ from the secretory to the nutrient side were almost the same. In the opposite direction the rate of transport of Na^+ was similar; the rate of transport of K^+ (whose concentration in the nutrient solution was only 4·1% of the Na^+ concentration) was 5% of the rate of Na^+ transport. Thus there was no appreciable directional asymmetry of the type observed in frog skin for which the influx was about ten times the outflux (Ussing, 1949).

Table 1. *Rates of transport of* Na^+ *and* K^+ *across resting isolated frog gastric mucosa*

Direction of transport ...	Secretory to nutrient side		Nutrient to secretory side	
Ion carrying activity	Na^+	K^+	Na^+	K^+
Concentration (mM)	120	120	110	4·5
No. mucosae studied	5	7	4	6
Rate of transport (μa./cm.^{2}):				
Mean	22	21	29	1·5
Range	10–39	10–35	20–39	0·8–2·6

1 μa. $= 6{\cdot}28 \times 10^{12}$ univalent ions/sec.
$= 1{\cdot}04 \times 10^{-8}$ mM univalent ion/sec.

In nine experiments with ^{42}K, the rate of transport of K^+ in both directions was reversibly increased up to sevenfold when histamine was added to the nutrient solution. This increase was not

dependent upon the initiation of acid secretion. A comparable effect of adrenaline on the rate of transport of Na^+ through frog skin was observed by Barker Jørgensen (1947) and Ussing (1949).

Experiments in which an applied electric current was passed through the mucosa in either direction showed that K^+ and/or Na^+ can carry at least part of the current (cf. Crane, Davies & Longmuir, 1948; Davies & Ogston, 1949). It is of interest that the electric currents which can be maintained in an external circuit by isolated frog gastric mucosa (Crane *et al.* 1948) are of the same order as the cation currents which continuously cross the mucosa (Table 1).

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Observations on the Organic Matrix of Bone. By H. J. Rogers. (*Biological Research Unit, Dental School and Hospital, Leeds* 1)

During the normal resorption of bone and in some pathological conditions such as hyperparathyroidism the organic matrix as well as the inorganic salts of the bone are removed (e.g. Burns & Henderson, 1946). Thus any understanding of the process of resorption must take into account the removal of the organic as well as the inorganic material. Moreover, such is the paucity of our knowledge of the biochemical mechanisms underlying the resorptive process that either the removal of the hydroxyapatite or of the integrating organic matrix may be the primary step. The present work is concerned with some of the constituents of the organic matrix of bone, their quantitative changes with age and their distribution in the bone.

The amount of total N, calcium, and phosphorus in ether-extracted dried samples of human bone powder has been measured. The amount of reducing substances and hexosamine has also been measured after acid hydrolysis of the powder. The samples studied were thoroughly cleaned pieces of femur taken from the centre of the shaft of bones obtained from people dying as the result of accidents. The results obtained show that the concentration of total nitrogen falls with age, a result anticipated by Roche & Garcia (1936) for rats and more recently by Strobino & Farr (1949) for oxen.

The amount of reducing substances (3·0–4·5% expressed in terms of glucose on dry bone powder) and of hexosamine (0·10–0·26% expressed as glucosamine-HCl on dry bone powder) also show a considerable reduction. The fall in concentration of both protein and 'polysaccharides' is greatest in the early years of life (12 months–14 years).

The nature of the polysaccharides and other 'reducing substances' present in bone is under investigation. Levene (1925) reported, without giving details, the isolation of a sulphated polysaccharide from bone, and Pincus (1948) claims the isolation of chondroitin sulphate from dentin. It has been found that during the process of decalcifying bone powder by cold 2N-HCl (3 hr. treatment at 25° C.) about 30% of the N and of 'polysaccharide' is rendered soluble.

Ground sections of bone have been stained by a modification of the Hotchkiss (1947) procedure for polysaccharides and high concentrations of staining material have been found in the Haversian canals and very low concentrations distributed in the matrix. Decalcified sections have yielded similar results.

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Preliminary Observations on the Metabolism of the Alveolar Bone of Mammals. By S. WEIDMANN and H. J. ROGERS. (*Biological Research Unit, Dental School and Hospital, Leeds* 1)

One of the most serious features of many types of paradontal disease (pyorrhea) is the loss of part of the alveolar bone which normally encases the roots of the teeth and thus holds them firmly in position. The bone may either be resorbed locally around one or more teeth, a condition which is often associated with deep bacterially infected pockets between the 'gum' and tooth, or, sometimes, a general horizontal resorption of the alveolus may appear to take place. A knowledge of the metabolism of this bone taken in comparison with that of members of the rest of the skeleton might, therefore, be of considerable value in helping forward our understanding of the general syndrome of pyorrhea.

The rate of exchange of radioactive phosphorus (^{32}P) has been investigated using cats. These animals were chosen for the preliminary work because the relative degree of calcification of the alveolus, cleaned by a flotation technique, was similar to that found for *Homo sapiens*. Cats suffer with a mild paradontal condition, but it was quite possible to select radiographically 'normal' animals, i.e. those which showed no apparent resorption of the alveolar bone on X-ray examination. A solution of Na_2HPO_4 containing marked phosphorus was injected subcutaneously and the cats killed by ex-sanguination under anaesthesia, after varying periods of time. The level of the radioactive element in the alveolar femur shafts and heads and in the blood serum was compared with the total concentration of phosphorus ($^{31}P + ^{32}P$) in the samples. In some experiments the level of radioactive phosphorus in the plasma was maintained approximately constant during the experimental period by repeated daily injections of the labelled salt solution; urine measurements were used to reflect the plasma level in such experiments.

The rate at which the phosphorus enters the bone salt of the trabecular alveolar bone is very much less than the corresponding rate for the trabecular bone of the epiphyses and little greater than that for the shaft of the long bone.

It is difficult to interpret this result by the hypothesis, so far unproved, that the exchange of phosphorus in the bones of the living animal is entirely due to the ionic exchange which takes place in powdered bone ash, and is thus a function of the degree of contact between the hydroxyapatite crystals and the circulating plasma (Neuman & Riley, 1940). If this were the whole explanation we should have to assume that the surface of crystals exposed in the trabeculae of the alveolar bone is only slightly different from that in hard bone, a conclusion difficult to accept in view of the histological and radiographic appearance of the bone.

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The 'Early-lag' Phase of *Aerobacter aerogenes*. By S. DAGLEY, E. A. DAWES and G. A. MORRISON. (*Department of Biochemistry, School of Medicine, Leeds*)

Working with an aerated synthetic glucose-ammonium medium, Lodge & Hinshelwood (1943) found that bacteria taken early in their logarithmic growth phase showed 'early lag' when inoculated into a fresh lot of medium of the same composition. Several hours after the end of the logarithmic phase, 'late lag' developed, whilst at some intermediate point there was a minimum in the curve relating lag and 'age'. We have shown that these relationships are greatly influenced by conditions both prior to and during growth in the basal medium. In the work summarized below, bacteria from a stock maintained either on slopes or in broth were subcultured three times in succession in the basal medium before inoculation into the medium in which growth was followed.

(1) Cells from a stock kept on slopes showed no early lag at ages for which the original workers (1943) demonstrated considerable lag, their strain being maintained by serial subculturing in broth. Slope-maintained cells, however, showed lag when harvested and washed.

(2) The same strain when kept for 6 weeks in broth showed marked early lag in unaerated media, falling sharply to zero about one-third of the way through the logarithmic phase. Beyond this point, i.e. whilst rapid cell division was still in progress, a later lag phase developed. Although, therefore, the position of the minimum in the lag-age curve has formerly been regarded as approximately coincident with the onset of the stationary phase, work with the present strain indicates that the two phenomena are not necessarily connected.

(3) Cells grown in unaerated media showed a few hours' lag at all ages when transferred to the synthetic medium through which air was bubbled, but this lag was practically abolished by three successive subcultures in the presence of the air stream. Cells which had been stored in broth, as in (2), and then trained in this way to optimum aerobic growth were used as inocula for both aerated and unaerated media. In both cases, although minimum lag was given towards the end of logarithmic growth, the lag-age curves were much shallower than in (2), where inocula were taken from unaerated cultures.

(4) Viable counts of cultures with early lag showed that in this phase cell division is not absent, but that there is a definite logarithmic rate of growth one-third to one-quarter the normal value. The latter is the rate which is measured by turbidimetric methods or by haemocytometer counts.

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Influence of Amino-acids, and Compounds in the Krebs Oxidation Cycle, on 'Early Lag'. By S. Dagley, E. A. Dawes and G. A. Morrison. (*Department of Biochemistry, School of Medicine, Leeds*)

Lodge & Hinshelwood (1943) obtained direct experimental evidence for the view that early lag in a synthetic medium is due to diffusion from the cells, at high dilution, of the intermediates necessary for rapid cell division to commence. If this view is accepted it is seen that, by studying the growth responses evoked by the addition of small amounts of various compounds, it may be possible to gain information about the nature of these essential intermediates. The following results were obtained with cells trained to the basal medium by three serial subcultures.

(1) Using inocula from a slope-maintained stock taken from the early logarithmic phase but showing no lag period (see Dagley, Dawes & Morrison, 1949), the addition to the basal medium of any of the following compounds separately, at concentrations of about 10 parts per million, raised the initial growth rate significantly above its normal value: methionine, glutamate, aspartate, α-ketoglutarate, oxalacetate, succinate. By the time that visible growth appeared, growth rates had in all cases reverted to normal, although faint turbidity was reached much earlier when the additions were made.

(2) With the exception of aspartate, lag produced by washing cells from slope stocks was also partly removed by these additions.

(3) Malate and fumarate did not produce this effect over the same region of concentration; fumarate was definitely inhibitory and malate slightly so.

(4) Early lag is a phase of reduced growth rate (Dagley *et al.* 1949). For cells exhibiting this phase, plate counts showed that the various additions at the concentrations stated raised this low rate of growth to a value approaching normal.

It is seen that whilst several compounds (and related amino-acids) taking part in the Krebs cycle produce growth responses, others do not. Any theory assigning to the Krebs cycle a place of importance in the economy of the bacterial cell must take this fact into account, as well as our observation that fluoracetate had little adverse effect on bacterial growth; iodoacetate, over the same concentration range, is a powerful inhibitor.

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Factors Influencing the Polysaccharide Content of *Escherichia coli*. By S. Dagley and E. A. Dawes. (*Department of Biochemistry, School of Medicine, Leeds*)

Cells grown on an amino-acid medium of known composition were washed, treated with 2 N-H_2SO_4 for a length of time known to ensure complete hydrolysis of the polysaccharide, and the resulting reducing sugar estimated by the Somogyi (1937) method. Expressing the results as mg. polysaccharide per 100 mg. dry bacterial weight a storage of about 7 % was obtained in the absence

of sugar, but when glucose, lactose or galactose were added the storage rose with concentration to about 18%, with a sugar concentration of 2 g./l. Dawson & Happold (1943) reported no storage with mannose and we find that additional storage with this sugar and with fructose is slight. Storage was studied when the various sugars were present at a pH range from 6 to 9. For glucose and galactose, which give good storage at pH 7·5, alkaline or acid conditions had a marked adverse effect on storage, which is probably due to the fact that under these conditions little sugar is consumed before the stationary phase is reached. Storage in the presence of mannose and fructose is not so dependent upon pH, the low value at adverse pH values being similar to that under favourable conditions.

In an attempt to elucidate the function of polysaccharide storage its value was determined in the various phases of growth of the bacteria both in the amino-acid medium and in a basal glucose-ammonium medium. During the stationary phase the storage was reduced by almost half during a period of 40 hr. It might be suggested that in this phase the cells were utilizing stored polysaccharide when foodstuffs in the medium were no longer available, but the following observations do not support this view. The fall in storage is not exactly coincident with the cessation of cell division and the same storage is obtained in the basal ammonium medium whether exhaustion of glucose determines the onset of the stationary phase or whether glucose is in excess and division ceases because the nitrogen source is exhausted.

The phases of growth were followed in this work by turbidity determinations and we have repeatedly confirmed the observation of Monod (1942) that when cell division ceases there is a definite fall in bacterial mass during the course of several hours. Although this might be ascribed to consumption of cell material, such as polysaccharide, during the resting phase this is not substantiated by the fact that the turbidity falls as soon as the stationary phase is reached, whereas the polysaccharide storage does not.

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Experiments on the Mobility of the Methyl Group in Dimethylacetothetin, $(CH_3)_2S^+.CH_2CO.O^-$.

By F. Challenger and Patricia Fothergill. (*Department of Chemistry, The University, Leeds*)

Willstätter (1902) showed that at about 300° betaine, $(CH_3)_3N^+CH_2COO^-$, is partly converted to methyl dimethylamino-acetate, and Challenger, Taylor & Taylor (1942) found that the mobile methyl group can be transferred by heat to compounds of S, Se and Te giving $(CH_3)_2S$, $(CH_3)_2Se$ and $(CH_3)_2Te$. With aromatic primary amines, $R.NH_2$, formation of $R.NHCH_3$ occurs and disulphides, $RS.SR$, also undergo methylative fission to $RSCH_3$. After the isolation of dimethyl-β-propiothetin chloride, $(CH_3)_2S^+(Cl^-)CH_2.CH_2COOH$, from *Polysiphonia fastigiata* by one of us and Simpson (1948), Maw & du Vigneaud (1948) found that this thetin salt and also the chloride of dimethylacetothetin, $(CH_3)_2S^+.CH_2COO^-$, supported growth of rats on a diet deficient in methyl donors but containing homocystine. The occurrence of biologically labile methyl in both these thetins was further demonstrated by Dubnoff & Borsook (1948), who showed that the chlorides could methylate homocysteine to methionine using liver or kidney preparations of rats, guinea pigs and hogs.

It was of interest to find whether the mobility of the methyl group in the thetins could be detected as in the case of betaine. We find that when the anhydride of dimethylacetothetin hydroxide, $(CH_3)_2S^+CH_2CO.O^-$, is heated with aniline, *p*-toluidine or β-naphthylamine the *N*-methyl compounds of these bases, $RNHCH_3$, are formed and characterized as the *N*-nitroso-derivatives, $RN(NO)CH_3$, or as the acetyl derivative. Much $(CH_3)_2S$ and CO_2 are evolved and considerable quantities of the diarylurea, $CO(NHR)_2$, are formed. Anilinoacetanilide, $C_6H_5NHCOCH_2NHC_6H_5$, was also isolated, a type of compound repeatedly observed in the betaine experiments, where however no diarylurea was detected. The methylations effected by betaine and thetin may involve a positive or a neutral methyl radical (Challenger, 1945, 1947). It is conceivable that the methylation of the amines may be effected by a neutral —CH_2— radical or even by the decarboxylation of an arylglycine. The diarylureas may perhaps arise from decomposition of a more complex compound or from the free radical

$\div\underset{\cdots}{\underset{O}{C}}\div$ (see below). Further work is in progress.

Possible reactions (compounds underlined have been detected):

$$(CH_3)_2S^+.CH_2CO.O^- + RNH_2 = \underline{(CH_3)_2S} + RNH.CH_2COOH,$$

$$R.NHCH_2COOH + RNH_2 = \underline{RNH.CH_2.CONHR} + H_2O,$$

$$RNHCH_2COOH = \underline{RNHCH_3} + \underline{CO_2},$$

$$(CH_3)_2S^+.CH_2COO^- + RNH_2 \rightarrow CH_3S.CH_2.COOH + \underline{RNHCH_3}$$

(transmethylation; no derivative of CH_3SCH_2COOH yet isolated),

$$(CH_3)_2S^+.CH_2COO^- = (CH_3)_2S + \div CH_2-C(=O)-O\div,$$

$$\div CH_2-C(=O)-O\div \rightarrow \div CH_2\div + \div C(=O)-O\div,$$

$$\div C(=O)-O\div + RNH_2 = RNH.CO.OH \xrightarrow{RNH_2} \underline{CO(NHR)_2} + H_2O.$$

Gaseous CO_2 and boiling aniline give no diphenylurea. The interconversion of the thetin and the isomeric ester, $CH_3SCH_2COOCH_3$, has not yet been effected.

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Carbohydrates Present in the Subterranean Organs of some Members of the Compositae.

By J. S. D. Bacon and J. Edelman. (*Department of Biochemistry, The University, Sheffield*)

Extracts made by disintegrating Jerusalem artichoke tubers with water in the Waring Blendor contained about 2 mg./ml. of reducing substances. About 100 mg./ml. of reducing sugar (estimated by the method of Miller & Van Slyke, 1936) was liberated on hydrolysis with 0·5 % oxalic acid at 100° for 30 min. Of this four-fifths was fructose (see Bacon & Bell, 1948) while the remainder was removed by the action of glucose oxidase (Keilin & Hartree, 1948) and was therefore glucose. Increasing concentrations of ethanol precipitated increasing amounts of polysaccharide material, but all fractions so obtained were soluble in cold water (cf. Thaysen, Bates & Green, 1929).

Examination of such extracts by the method of paper partition chromatography (Partridge, 1948) showed the presence of small amounts of free fructose and of a series of components containing fructose with R_F values (in three solvent mixtures) ranging from those for sucrose to zero. In chromatograms run for 2–5 days with butanol-acetic/water mixture at least six such components could be seen in addition to an undifferentiated zone of R_F below 0·02.

A similar series of components was found in extracts of the subterranean organs of five other species of Compositae.

One of the components has been tentatively identified as sucrose by:

(1) The R_F values in three solvents.

(2) The lack of reducing power as judged by the use of $AgNO_3$ and benzidine sprays.

(3) Its disappearance from chromatograms of tuber extracts treated with an invertase preparation from yeast.

(4) Extraction from the paper and estimation of fructose and total reducing sugar after mild acid hydrolysis.

(5) Hydrolysis by invertase *in situ* on a two-dimensional chromatogram and subsequent identification of glucose and fructose.

Similar methods have been applied to the examination of the other components of the extract. Additional information as to their composition has been obtained by the use of the enzyme preparation described in the following abstract.

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The Action of an Enzyme Preparation from *Helianthus tuberosus* L. (Jerusalem artichoke) on Carbohydrates Present in the Tubers. By J. EDELMAN and J. S. D. BACON. (*Department of Biochemistry, The University, Sheffield*)

Green (1887) described an enzyme hydrolysing inulin in extracts of tubers of the Jerusalem artichoke, which he named inulin-ferment. Other authors have studied inulases from plants, e.g. *Cichorium Intybus* L. (chicory) (Wolff & Geslin, 1918) and from moulds (see Oppenheimer & Pincussen, 1929).

Extracts of the tubers made as described in the previous abstract showed on incubation a marked negative change in optical rotatory power which could be correlated with an increase in reducing substances. The quantitative relations were consistent with the hypothesis that fructose was being liberated from material having a negative rotation. No such change was observed in boiled extract. The activity was greatest at about pH 5.

Attempts were made to concentrate and purify the enzyme activity. The standard substrate solution used was a boiled extract of tubers, adjusted to pH 5·0 with acetate buffer and filtered. The most active preparation obtained liberated about 9 mg. of reducing sugar when 0·5 ml. was added to 2·5 ml. of standard substrate solution and incubated for 30 min. at 40°. This represents a 20-fold concentration.

Paper partition chromatography (Partridge, 1948) has been used to separate the components of the standard substrate solution. The action of the enzyme preparation upon these and upon inulin was investigated. Both glucose and fructose are among the products of hydrolysis and intermediates have been detected resembling in R_F value the components of the freshly extracted juice (see preceding abstract). The rate of sucrose hydrolysis was only about one-twentieth of that of the standard substrate under the test conditions.

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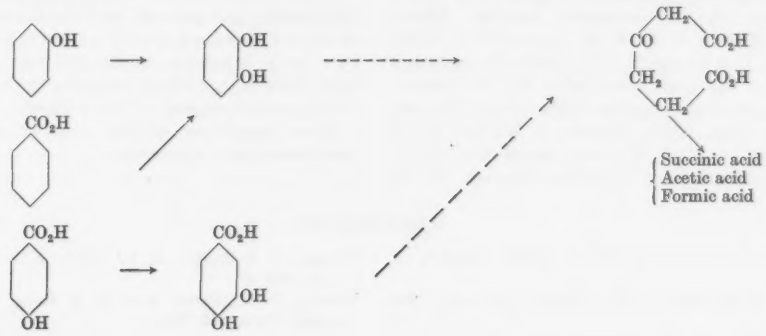
The Mechanism of the Bacterial Oxidation of Certain Aromatic Compounds, together with the Preparation and Properties of a Cell-free Enzyme System which Accomplishes Ring Cleavage. By W. H. PARR, R. A. EVANS and W. C. EVANS. (*Biochemical Laboratories, University College of Wales, Aberystwyth*)

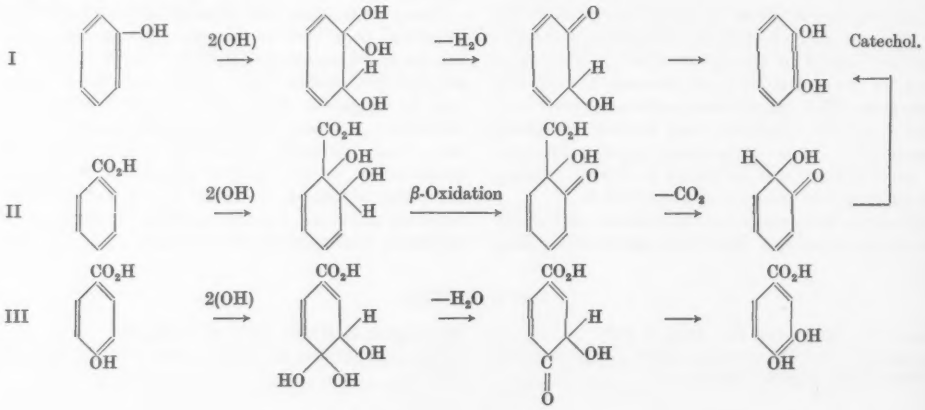
It has been established that vibrio O1 (Happold & Key, 1932) oxidizes the following aromatic compounds through the following intermediates (Evans & Happold, 1939; Evans, 1947; Kilby, 1948; Evans, Parr & Evans, 1949):

By the application of the simultaneous adaptation technique, Stanier, Sleeper, Tsuchida & Macdonald (1949) have concluded that *Pseudomonas fluorescens*, capable of metabolizing these substrates, does so in a similar manner. This we have confirmed,



by chemical isolation of intermediates, working with their organisms. An important point, brought out by the Stanier technique, is the essentially distinct pathways of benzoic and *p*-hydroxy benzoic acids, during the initial stages of their metabolism (Stanier *et al.* 1949).

To account for the intermediates isolated, we suggest the following mechanisms for the oxidations —they are visualized as occurring by the aid of H_2O_2 (or OH-free radicals):



In a similar manner, the stages leading to ring cleavage with the production of β-keto adipic acid may be visualized.

We have been successful in the preparation of the cell-free ring-splitting enzymes, by the powdered pyrex glass grinding method. Some properties of this system, including the effect of inhibitors, were discussed.

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Some Observations on the so-called 'Bracken Poisoning' of Farm Animals, and the Aneurin Inactivating Mechanism of Various Plants. By H. E. Roberts, E. T. R. Evans and W. C. Evans. (*University College of Wales, Aberystwyth*)

We have already confirmed the presence of a mechanism in the common bracken (*Pteris aquilina*) (Evans, E. T. R. & Evans, W. C. 1949; Evans, W. C. & Evans, E. T. R. 1949) which renders aneurin in the diet unavailable to the animal. These experiments, together with others (Weswig, Freed & Haag, 1946; Thomas & Walker, 1949), were done on rats. We now report the experimental production of 'bracken staggers' in the horse, together with observations on the condition. The aneurin and pyruvic acid blood levels of such an animal presents a typical picture of avitaminosis B_1. Daily injections of 50–100 mg. of aneurin, subcutaneously, without changing the bracken diet led to a rapid recovery of the animal.

Some properties of the aneurin inactivating mechanism were discussed.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **280th Meeting** *of the Biochemical Society was held in the Biochemistry Department, King's College, Strand, London, W.C. 2, on Saturday 22 October 1949, when the following papers were read:*

COMMUNICATIONS

Preformed Vitamin A in Northern Krill. By S. K. Kon and S. Y. Thompson. (*National Institute for Research in Dairying, University of Reading*)

Wagner (1939) claims to have extracted 14·5 mg. of pure β-carotene from 1 kg. of Crustacea ('Gattung der Euphansia superba Dana') forming the food of Blue and Fin whales caught at the Lopra whaling station in the Faeroes. It is generally accepted that astaxanthin pigments are the main carotenoids of Crustacea and that β-carotene is present, if at all, only in small amounts. Blue and Fin whales live in northern waters largely on two euphausiids: *Meganyctiphanes norvegica* and *Thysanoessa inermis* (cf. Einarsson, 1945). With the generous help of the Marine Station at Millport, Isle of Cumbrae, we obtained specimens of *Meganyctiphanes norvegica*, of *Thysanoessa raschii*, closely allied to *T. inermis*, and of several other species of Crustacea all from Loch Fyne. A further mixed sample of *Meganyctiphanes norvegica* with small plankton and a sample of the common shrimp, *Crangon vulgaris*, were kindly supplied by the Marine Biological Laboratory at Aberdeen.

Our observations with *Meganyctiphanes norvegica* agree with those already reported on *Euphausia superba* Dana, the Krill of antarctic whales (Thompson *et al.* 1949; Kon & Thompson, 1949), and indicate that, contrary to Wagner's (1939) claims, those whales feeding on larger Crustacea derive their vitamin A from preformed vitamin A and not from carotene. A whale's stomach may contain up to 1200 l. of Krill (Einarsson, 1945), and such enormous quantities could, no doubt, account for the richness of whale liver in vitamin A. The origin of these stores has so far been in doubt because of conflicting evidence about the presence of vitamin A and active carotenoids in zooplankton.

		Vitamin A		Carotenoids: Total*		
Species	Locality	i.u./g. shrimp	i.u./g. oil	μg./g. shrimp	μg./g. oil	β-Carotene
Meganyctiphanes norvegica	Loch Fyne	15	680	42	1900	Faint trace
Thysanoessa raschii	Loch Fyne	32	495	33	500	Faint trace
Pandalus bonnieri	Loch Fyne	2·1	89	24	1000	Faint trace
Spirontocarus spinus	Loch Fyne	1·0	22	27	950	Trace
Crangon allmanni	Loch Fyne	0·4	30	5	390	Trace
Meganyctiphanes norvegica, mixed sample with other plankton	Neighbourhood of the Faeroes	1·6	145	23	2100	Faint trace
Crangon vulgaris	Bay of Holland, Stronsay, Orkneys	0·2	21	5	550	Trace

* Read at the absorption maximum for β-carotene and calculated as such.

The Crustacea were identified and placed in absolute alcohol immediately after netting. After extraction of the fat with light petroleum and saponification, chromatographic separation and measurement of vitamin A and β-carotene were done as described by Thompson (1949) and Thompson, Ganguly & Kon (1949).

None of these Crustacea contained more than traces of β-carotene, but, as shown in the table, relatively large quantities of preformed vitamin A, mostly in the ester form, were found in *Meganyctiphanes norvegica* from Loch Fyne and in *Thysanoessa raschii*. The vitamin A fraction gave a typical Carr-Price reaction, and the potency calculated on this basis agreed with that obtained from the ultraviolet absorption curve corrected for irrelevant absorption. The fraction was further identified by its yellow fluorescence in ultraviolet light and by mixed chromatography of the original extract with vitamin A ester and, after saponification, with vitamin A alcohol.

Our grateful thanks are due to Dr E. Ford for all the facilities at Millport, to Dr A. P. Orr for his generous and kind help there in the collection of specimens, to Dr C. E. Lucas for samples and to Dr J. A. Lovern for constant interest and advice.

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Naturally Occurring Organo-silicon Compounds. By P. F. Holt and Deirdre M. Yates. (*University of Reading*)

The separation of an alcohol-soluble fraction of plasma in which were silicon-containing compounds was described by Holzapfel (1943). Ohlmeyer & Olpp (1944) described a procedure for the determination of the organic silicon compounds in tissues which involves their extraction from the tissues by ether-alcohol, ignition and fusion of the extract with sodium carbonate and the colorimetric determination of the silicon.

In attempting to fractionate the alcohol extract of liver and egg, no agreement could be obtained between the quantity of silicon in the alcoholic extract and the sum of the quantities in the fractions. It would appear that, using the method of fusion in an open crucible, a loss is incurred because of the volatility of the organo-silicon compounds. By applying the technique of the Parr bomb, combustion is effected without loss of volatile substances. Values obtained on an extract of egg yolk were higher and much more consistent than those obtained by the method of Ohlmeyer & Olpp.

Method. Water is abstracted from the tissue by the Dean and Stark procedure (Warne, 1942), their apparatus being modified to handle larger quantities of material.

The dry tissue is extracted under reflux with ethanol for 10–20 hr. (Experiments on chicken liver indicated that extraction is complete after about 6 hr.) The ethanol solution is filtered and the filtrate is evaporated to a brown fatty residue. Approximately 500 mg. of this residue is transferred to a nickel Parr micro-bomb, the crucible of which is half-filled from a mixture of 0·2 g. finely powdered sucrose and 6 g. sodium peroxide. The mixture is pressed hard into the bomb which is then closed and ignited, applying a small flame from an oxygen-coal gas hand blowpipe to the base of the crucible. The bomb is cooled, unsealed and dropped into water in a plastic beaker. The silica is determined colorimetrically in an aliquot of the resulting solution after separation of phosphate by the method of King (1939).

Results. Since the identity of the silicon compounds is unknown, it is not possible to determine the accuracy of the method by making known additions to a tissue extract and comparing the recovery values. However, a number of estimations has been made on tissue extracts by both the bomb method and that of fusion in an open crucible. Whilst the former method gives reasonably consistent values, the latter gives figures invariably lower than those obtained with the bomb and extremely variable. Examples of figures obtained with an extract of egg yolk are given in Table 1.

Table 1. *Silica content of extract of egg yolk*

(mg. SiO_2 per 100 g. extract.)

Alkaline fusion	Bomb method
16·1	29·4
11·1	30·7
1·9	30·1
14·0	29·8
7·5	37·1
17·4	32·3

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The Quantitative Analysis of Ribonucleic Acids. By R. Markham and J. D. Smith. (*Agricultural Research Council Plant Virus Research Unit, Molteno Institute, Cambridge*)

The recently developed methods of analysis of purines and pyrimidines by paper partition chromatography (Hotchkiss, 1948; Vischer & Chargaff, 1948; Markham & Smith, 1949) have made it possible to detect and estimate minute quantities of the free bases and their ribosides. The application of these techniques to the analysis of ribonucleic acids has, on the other hand, been complicated by the lack of a satisfactory method of hydrolysis for freeing the pyrimidines or their nucleosides quanti-

tatively, and little reliance can be placed on published values for the pyrimidine content of these substances. On the other hand, hydrolysis by N-hydrochloric acid at 100° for 1 hr. releases purines as such and the pyrimidines as nucleotides quantitatively. We have found it possible to resolve this mixture in several ways using two dimensional chromatography or several runs in different solvents, but, with a view to simplifying the method as much as possible, we have investigated the effects of the alteration of water content and acidity on solvent systems based on the simple alcohols. Using this information we have designed a solvent which will resolve guanine, adenine, cytidylic acid and uridylic acid on a single dimensional chromatogram. With this new technique it is possible to make a complete quantitative base analysis on as little as 0·1 mg. of ribonucleic acid.

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The Absorption of Vitamin A. By Eva Eden and K. C. Sellers. (*Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council; and the Veterinary Investigation Centre, Institute of Animal Pathology, Cambridge*)

The absorption of vitamin A by the lymph stream has been demonstrated in several species (see previous communication, Eden & Sellers, 1949). Earlier studies on the mechanism of vitamin A absorption by Gray, Morgareidge & Cawley (1940) showed that in the rat vitamin A esters were hydrolysed in the intestinal tract. To our knowledge, the changes of the form of vitamin A during absorption have not been examined in ruminants, hence it was decided to repeat and extend the work of Gray *et al.* (1940), using sheep and bovines of varying ages as experimental animals.

Twenty animals were dosed orally with vitamin A alcohol or ester (Distillation Products Ltd.), emulsified in reconstituted separated milk. The dose of vitamin A was adjusted to 5000 i.u./kg. of body weight and the animals were slaughtered 4 hr. after dosing. This was the time interval chosen, as previous experiments (Eden & Sellers, 1949) had shown that, although the rate of absorption of vitamin A was variable, it usually reached its maximum at this time. The concentrations of vitamin A alcohol and ester were estimated in the blood before and after dosing. Estimations were also made on slaughter in the intestinal contents and wall and in the lymph. Vitamin A alcohol and ester were separated chromatographically using a modified method of Hoch & Hoch (1946). (Further details of the technical procedure will be published later.)

Four hours after the administration of vitamin A esters almost complete hydrolysis had occurred in the intestinal lumen of some bovines, whereas in others hydrolysis was only partial. Estimations performed on the intestinal mucosa at the same time showed that about 75% of the absorbed vitamin A was in the ester form, and the same was true in animals dosed with vitamin A alcohol. These results indicate that the intestinal wall is capable of esterifying free vitamin A alcohol, but so far we have been unable to obtain evidence that esterified vitamin A is absorbed in its unaltered form. After leaving the intestinal wall, the absorbed vitamin A apparently remained esterified, as the rise of the vitamin in the intestinal lymph following administration of either form was mainly present in the ester form. Confirmatory results have also been obtained in experiments performed on sheep and newborn calves.

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Further Studies of the Biogenesis of Porphyrins in Rabbits. By HELEN M. MUIR and A. NEUBERGER. (*National Institute for Medical Research, Hampstead, N.W.* 3)

The specific incorporation of the nitrogen of ^{15}N-labelled glycine into haemin has been clearly demonstrated by Shemin & Rittenberg (1946), and it has also been shown that the isotope is equally distributed between both types of pyrrole ring (Muir & Neuberger, 1949; Wittenberg & Shemin, 1949). Of the two carbon atoms of glycine only the methylene carbon atom appears in the porphyrin molecule (Grinstein, Kamen & Moore, 1948; Altmann, Casarett, Masters, Noonan & Salomon, 1948). Rittenberg has also identified acetate as a precursor of porphyrins (Bloch & Rittenberg, 1945; Radin, Rittenberg & Shemin, 1949).

In the present investigation rabbits were injected with the following three isotopic compounds:

$^{15}NH_2{}^{14}CH_2.COOH$; $^{14}CH_3.COOH$; $CH_3.{}^{14}COOH$.

The blood porphyrin was isolated and degraded by oxidation by methods previously described (Muir & Neuberger, 1949). The relative activities of the three fragments methylethylmaleic acid imide, derived from rings I and II, haematinic acid from rings III and IV and CO_2 mainly derived from the methyne carbon atoms are shown in Table 1. It can be seen that with both the acetates the two imides account for the whole of the activity of the mesoporphyrin, but with glycine only 65 %. This and the relative activities of the carbonate fraction indicate clearly that most if not all the methyne bridge carbon atoms are derived from the original glycine. The $^{14}C/^{15}N$ ratio in the porphyrin, as compared with that of the glycine, showed that there are about 2·4 times as many carbon atoms derived from glycine as nitrogen atoms. If it is assumed that the four N atoms of porphyrin come from glycine and that no extensive reversible deamination of glycine takes place, it follows that all four methyne carbon atoms and at least as many of the ring carbon atoms are derived from the methylene group of glycine.

In experiments using isotopic acetates, the utilization of the methyl group of methyl-labelled acetate is found to be considerably more efficient than the utilization of the carboxyl group of carboxyl-labelled acetate. Furthermore, the distribution of the carbon atoms from these two precursors is not the same. In the case of methyl-labelled acetate, both kinds of pyrrole ring are equally radioactive, but after injecting carboxyl-labelled acetate, the acidic pyrroles from rings III and IV are considerably more radioactive than those from rings I and II, which are not acidic. It was also possible to exclude both the acetates from being direct precursors of the methyne carbon atoms. The implications of these results were discussed.

Table 1

Substance administered	Specific activities (counts/min./μ mole) Mesoporphyrin	Haematinic acid	Imide	$BaCO_3$
$^{15}NH_2{}^{14}CH_2COOH$	107·5 ±1·75	17·52±0·27	17·39±0·23	9·9 ±0·3
$CH_2{}^{14}COOH$	32·35±1·09	12·66±0·31	2·97±0·15	0·44±0·33
$^{14}CH_3COOH$	27·9 ±0·67	7·55±0·25	6·5 ±0·22	0·08

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A Modified Chemical Estimation of Nicotinic Acid. By D. E. HUGHES. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, The University, Sheffield* 10)

Nicotinic acid reacts with cyanogen bromide to form a yellow-coloured derivative of glutaconic aldehyde (König, 1904), which can be estimated photometrically either directly (Kokovikhyna, 1946) or after condensation with various amines (Harris & Raymond, 1939). It was found that neither method was satisfactory for estimating accurately small quantities of nicotinic acid (10–25 mμmol.). This was due to low light absorption of the aldehyde or to instability of the amine derivatives.

Treatment of the glutaconic aldehyde derivative with NaOH resulted in the formation of material

with a strong absorption in the near ultra-violet (compare Larsen & Haag (1944) and Chaudhuri & Kodicek (1949)). The peak of the absorption curve was at 372 mμ. The $\epsilon^{\text{max.}}_{372\,m\mu} = 3{\cdot}02 \times 10^4$ (maximum molar extinction coefficient on the basis of nicotinic acid). The absorption obeyed Beers's law with quantities of nicotinic acid ranging from 0·625 to 100 mμmol. in a total volume of 3·0 ml.

The method of estimation was as follows: fresh CNBr (Kodicek, 1940) is diluted with an equal quantity of buffer (100 ml. 0·5 M-phosphate buffer pH 6·0, and 100 ml. 0·5 M-acetate buffer pH 5·0). The pH of the solution is adjusted to 5·5–5·6 with N-NaOH or 10% acetic acid and checked with a glass electrode. The pH of the unknown nicotinic acid solution, as adjusted to pH 5·3–5·8 and 0·5 ml. is added to 1 ml. of the CNBr reagent. The mixture is heated at 60° for 20 min. and then cooled. Next are added 0·5 ml. of 6 N-NaOH and 1·0 ml. of water. The solution is left for 45 min. in the dark at room temperature, and the absorption is measured in the Beckmann spectrophotometer (model DU). The readings increase some 10% in the next 18 hr. after which they reach a maximum value. Duplicate samples with added nicotinic acid are run at the same time to eliminate the effect of salt concentration and pH.

Nicotinamide, nicotinamide ribose phosphate and cozymase gave an absorption equal to 16·5% of that of nicotinic acid at 372 mμ., but the peak was at 352 mμ. ($\epsilon_{352\,m\mu} = 1{\cdot}16 \times 10^4$). Aneurin gave an absorption peak at 372 mμ. ($\epsilon_{372\,m\mu} = 0{\cdot}92 \times 10^4$). Nicotinyl hydrazide and nicotinuric acid behaved as nicotinic acid while trigonelline, nicotinamide-N^1-methylchloride, pyridoxine, pyridoxal and pyridoxamine did not absorb between 400 and 340 mμ.

The method was used to estimate nicotinic acid in solutions containing glucose and salts and in acid hydrolysates of *Lactobacillus arabinosus*. The results were in good agreement with those of the microbiological method. The method also promises to be useful for estimating separately nicotinamide and nicotinic acid in solutions containing both.

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An Improved Medium for Microbiological Assays with *Lactobacillus casei*. By E. Kodicek and S. P. Mistry. (*Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*)

The requirements of *Lactobacillus casei* for the individual constituents present in synthetic media have been studied and a modified medium* has been devised which can be used for a number of vitamin assays including pteroylglutamic acid, riboflavin, nicotinic acid, biotin, pantothenic acid, strepogenin and also for the estimation of tryptophan.

The medium has been developed specifically for the estimation of pteroylglutamic acid. It was found that an overall increase in the concentration of B-vitamins was essential for obtaining a maximum acid production of 28–30 ml. 0·1 N-acid per 10 ml. of basal medium which is almost equal to the theoretical yield of acid which could be produced from 3% glucose. A new salt mixture has been added and 3% potassium acetate was used instead of the sodium salt. The medium has been employed for routine assays of nicotinic acid and of strepogenin (Kodicek & Mistry, 1949). The estimation of nicotinic acid using *L. casei* was found to give more reproducible results than were obtained with the previous method employing *L. arabinosus* (Kodicek & Pepper, 1948). Only solutions of the other crystalline vitamins have been tried and extracts have not been assayed so far.

The new medium has been compared with media used by other workers and has been found to give more satisfactory results. It contains acid hydrolysed casein, which is a more reproducible constituent than the enzymatic digest used by Roberts & Snell (1946). Furthermore, when assaying strepogenin activity, enzymatic digests of casein could not be used because of their strepogenin content and so far no methods are available to free them completely of this factor.

* *Composition of basal medium, per* 10 *ml.*: glucose and potassium acetate (hydrated), 300 mg. each; K_2HPO_4 and KH_2PO_4, 25 mg. each; salt solution E (Kodicek & Pepper, 1948) 0·05 ml.; 'Labco' vitamin-free casein, H_2SO_4-hydrolysed, 50 mg.; norite-treated 'Difco' peptone, 2 mg.; DL-tryptophan and L-cystine, 2 mg. each; DL-alanine, 1 mg.; adenine, guanine, uracil and xanthine, 0·1 mg. each; aneurine, riboflavin, nicotinic acid and calcium D-pantothenate, 10 μg. each; pyridoxine, 25 μg.; *p*-aminobenzoic acid, 2 μg.; pteroylglutamic acid, 50 mμg.; biotin (free acid), 40 mμg. (Norite-treated peptone omitted for biotin, strepogenin and tryptophan assays. L-Asparagine, 1 mg., included for strepogenin assay.)

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Cozymase Synthesis by *Lactobacillus arabinosus* 17–5. By D. E. Hughes and D. H. Williamson. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, The University, Sheffield* 10)

The rate of anaerobic glycolysis by washed suspensions of nicotinic acid deficient *Lactobacillus arabinosus* is increased from two to fourfold by the addition of nicotinic acid, nicotinamide or cozymase (McIlwain, 1949). The acceleration of glycolysis is due to the increase of cellular cozymase (McIlwain, Stanley & Hughes, 1949) which is relatively stable in this organism (McIlwain & Hughes, 1948). For these reasons *Lb. arabinosus* has been used in the following preliminary studies on the bacterial synthesis of the cozymase. Cozymase and nicotinic acid were estimated by the methods previously outlined (McIlwain & Hughes, 1948). Glycolysis by washed suspensions of cells was followed manometrically. Cozymase synthesis was negligible in the absence of glucose.

The maximum rate of synthesis occurred in cells harvested after 16–18 hr. growth on the medium of Barton-Wright (1946), deficient in nicotinic acid ($1{\cdot}67 \times 10^{-7}$ M). Cells harvested earlier were not deficient and cells harvested later were deficient but synthesized cozymase more slowly. With cells grown for longer than 18 hr. the rate of glycolysis also falls but not to such a great extent as cozymase synthesis. The addition of 10^{-6} M-nicotinic acid to old cells (44 hr. growth) resulted in a maximum stimulation of glycolysis of 300 % after a lag period of 40 min. If nicotinic acid was replaced by equimolar amounts of nicotinamide or cozymase the lag period before the attainment of maximum glycolysis was 10–15 min. It would seem therefore that the system responsible for amidation of nicotinic acid has decayed at a faster rate than other systems involved in the synthesis of cozymase.

At nicotinic acid concentrations below 4×10^{-5} M the rate of cozymase synthesis was reduced. At higher concentrations the maximal rate of synthesis was maintained for only the first 10–15 min. and then fell progressively until the cellular concentration of coenzyme reached an average of 12·0 mμmol./mg. dry wt. cells. The final rate of synthesis was from 1·5 to 3·0 mμmol./mg. dry wt./hr. which was sufficient to replace a cozymase-like substance lost from the cells. None of the constituents of the growth medium affected either the rate of synthesis or the level of cozymase in the cells. Cells grown with adequate amounts of nicotinic acid (2×10^{-4} M) synthesized cozymase from nicotinic acid at a low rate (< 3·0 mμmol./mg. dry wt./hr.) compared with cells grown on the nicotinic acid deficient medium whose maximum rate of synthesis 24 mμmol./mg. dry wt./hr. Since these organisms do not break down cozymase at a rate comparable to the rate of synthesis (see McIlwain & Hughes, 1948) both the rate of synthesis and the level of cozymase in the cell appears to be controlled by a mechanism which inhibits synthesis as the cells approach saturation with cozymase.

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Inactivation of Serum Alkaline Phosphatase by Adrenaline and Related Substances. By A. B. Anderson. (*Biochemical Laboratory, North Middlesex Hospital, London, N.* 18)

Using sodium β-glycerophosphate as substrate in glycine buffer pH 9·4 (Jenner & Kay, 1932) and serum of high alkaline phosphatase content as the source of enzyme, it was found that adrenaline in a concentration of 5×10^{-4} M inhibited the phosphatase to the extent of 70–80 %. Adrenochrome in a concentration of $2{\cdot}5 \times 10^{-4}$ M gave 100 % inhibition. An equivalent inhibition, i.e. 70 %, was found with strong oxidizing agents such as potassium permanganate, as has been reported by Sizer (1942). Of the other phenolic compounds investigated only pyrogallol gave an equivalent inhibition of 70 % at

a concentration of 5×10^{-4} M. Slight inhibition was found with catechol 9%, quinhydrone and quinol 13% and *p*-quinone 22% at this concentration, while tyrosine, tyramine, resorcinol and ephedrine did not inhibit at all.

In view of the reported dependence of phosphatase activity on oxidation reduction potential (Sizer, 1942) measurements of the E_h of the complete reaction mixtures were made immediately after the addition of the inhibitors and again after 30 min. incubation. No correlation was found between E_h and degree of inhibition.

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The Inhibition of Plant Phosphorylases by β-Amylase and the Detection of Phosphorylase in Barley. By HELEN K. PORTER. (*Research Institute of Plant Physiology, Imperial College of Science and Technology, London, S.W. 7*)

Extracts (1 ml. H_2O to 1 g. ground grain) of ungerminated barley (BE) slowly convert glucose-1-phosphate (GlP), after a lag of about 4 hr., to an ester resistant to hydrolysis in 7 min. by N-acid. Very small amounts of free phosphate (P) are simultaneously released. When either potato or broad bean phosphorylase is included in a 1 ml. digest at pH 6·8 containing 0·1 ml. BE, 0·035 M-GlP and 200 mg.% soluble starch (s.p.) no P is released in the 1–2 hr. required for the reaction yielding amylose to reach equilibrium in the absence of BE. This inhibition is reduced and finally eliminated by continuous dilution of BE, and is removed by heat. Crystalline β-amylase behaves in the same way as BE towards added phosphorylase indicating that β-amylase (β) is the inhibiting agent in BE. Under the same conditions wheat, oat and rye extracts inhibit the bean enzyme to the extent of 70–80%.

If s.p. is exposed to the action of BE, its capacity to prime the phosphorylase reaction, measured by the amount of P released in 2 hr. following inactivation of β by heat, is reduced by 25% after 90 min. exposure, and by 40% after 24 hr. In the unheated system containing potato or bean enzyme there is thus some primer present for many hours, and if these enzymes were operative, release of P should be observed. It is concluded that β is inhibiting phosphorylase action and by some mechanism other than primer destruction. This view is supported by experiments using amylose glycollate, pretreated with β (AG), instead of s.p. Here potato enzyme effects release of only traces of P in 44 hr., although the priming capacity of AG is unimpaired as shown by subsequent heat inactivation of BE and further addition of potato enzyme.

Phosphorylase has been detected in BE by employing $(NH_4)_2SO_4$ to lower the ratio of β to phosphorylase to a level where inhibition is only partial. Throughout extraction and fractionation pH is maintained at 6·8 to avoid phosphorylase loss. Three fractionations of the precipitate formed between 40 and 50% saturation yield a preparation which liberates P from GlP relatively rapidly, and which with an achroic primer forms a polysaccharide giving a weak blue stain with iodine, after 5–10 min. This iodine staining material persists in the digest for 20–30 min. Finally, addition of starch and P to the preparation leads to formation of GlP.

The gift of samples of AG and crystalline β-amylase by Prof. S. Peat and Prof. A. K. Balls is gratefully acknowledged.

FORTHCOMING PAPERS

It is hoped to publish the following papers in the next issue of the *Biochemical Journal*:

The swelling of collagen in alkaline solutions. 1. Swelling in solutions of sodium hydroxide. By JOANE H. BOWES and R. H. KENTEN

A study of the peptides of cystine in partial hydrolysates of wool. By R. CONSDEN and A. H. GORDON

The respiration of rat-liver slices in the presence of some aliphatic amines, hydroxy amines and quaternary ammonium salts. By C. LONG

The reaction of oxidizing agents with wool. 1. The division of cystine into two fractions of widely differing reactivities. By P. ALEXANDER, R. F. HUDSON and M. FOX

The utilization of non-protein nitrogen in the bovine rumen. 5. The isolation and nutritive value of a preparation of dried rumen bacteria. By M. L. MCNAUGHT, J. A. B. SMITH, K. M. HENRY and S. K. KON

The utilization of non-protein nitrogen in the bovine rumen. 6. The effect of metals on the activity of the rumen bacteria. By M. L. MCNAUGHT, E. C. OWEN and J. A. B. SMITH

The turnover of radioactive phosphate injected into the subarachnoid space of the brain of the rat. By O. LINDBERG and L. ERNSTER

The determination of *N*-methyl-2-pyridone-5-carboxylic acid in human urine. By W. I. M. HOLMAN and D. J. DE LANGE

Studies in the biochemistry of micro-organisms. 81. The colouring matters of *Penicillium islandicum* Sopp. Part 2. Chrysophanic acid, 4:5-dihydroxy-2-methylanthraquinone. By B. H. HOWARD and H. RAISTRICK

Changes in water and ion metabolism and in kidney functions during the development of oedema in rats fed on protein-deficient diets. By S. E. DICKER

Pigments derived from tryptophan: (1) urorosein, (2) tryptochrome. By W. R. FEARON and W. A. BOGGUST

The oxidation of manganese by peroxidase systems. By R. H. KENTEN and P. J. G. MANN

Some properties of the insulin core. By J. A. V. BUTLER, D. M. P. PHILLIPS, J. M. L. STEPHEN and J. M. CREETH

Studies on the composition of horse oil. 1. The composition of horse oil in relation to the depot fats on other pasture-fed animals. By E. G. BROOKER and F. B. SHORLAND

Bile pigment precursors in normal human erythrocytes. By C. GARDIKAS, J. E. KENCH and J. F. WILKINSON

The action of enzymes on chlorophenylalanines. By H. BLASCHKO and JEAN STIVEN

The assimilation of amino-acids by bacteria. 9. The passage of lysine across the cell wall of *Streptococcus faecalis*. By V. A. NAJJAR and E. F. GALE

Properties of brain pyrophosphatase. By J. J. GORDON

Are phospholipins transmitted through the placenta? By G. POPJÁK and MARIE-LOUISE BEECKMANS

In search of a phospholipin precursor. By G. POPJÁK and HELEN MUIR

The effect of adrenal cortical preparations added *in vitro* upon the carbohydrate metabolism of liver slices. 1. The effect of adrenal cortical extract (eschatin) upon synthesis of glycogen and of total carbohydrate. By C. Y. CHIU and D. M. NEEDHAM

The effect of adrenal cortical preparations added *in vitro* upon the carbohydrate metabolism of liver slices. 2. The effect of some pure steroids upon carbohydrate synthesis, oxygen uptake and non-protein nitrogen. By C. Y. CHIU

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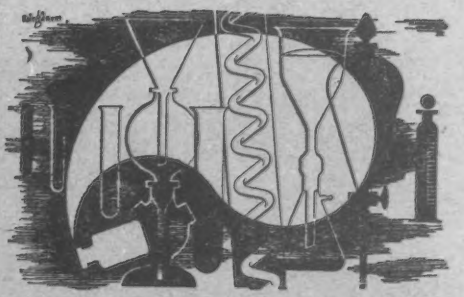

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